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Microalgas

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PUBLICACIONES

The effect of primary treatment of wastewater in high rate algal pond systems: Biomass and bioenergy recovery.

Bioresour Technol. 2019 May;280:27-36

Authors: Arashiro LT, Ferrer I, Rousseau DPL, Van Hulle SWH, Garfí M

Abstract

The aim of this study was to assess the effect of primary treatment on the performance of two pilot-scale high rate algal ponds (HRAPs) treating urban wastewater, considering their treatment efficiency, biomass productivity, characteristics and biogas production potential. Results indicated that the primary treatment did not significantly affect the wastewater treatment efficiency (NH₄⁺-N removal of 93 and 91% and COD removal of 62 and 65% in HRAP with and without primary treatment, respectively). The HRAP without primary treatment had higher biodiversity and productivity (20 vs. 15 g VSS/m²d). Biomass from both systems presented good settling capacity. Results of biochemical methane potential test showed that co-digesting microalgae and primary sludge led to higher methane yields (238-258 mL CH₄/g VS) compared with microalgae mono-digestion (189-225 mL CH₄/g VS). Overall, HRAPs with and without primary treatment seem to be appropriate alternatives for combining wastewater treatment and bioenergy recovery.

Combined resistance to oxidative stress and reduced antenna size enhance light-to-biomass conversion efficiency in *Chlorella vulgaris* cultures.

Biotechnol Biofuels. 2019;12:221

Authors: Dall'Osto L, Cazzaniga S, Guardini Z, Barera S, Benedetti M, Mannino G, Maffei ME, Bassi R

Abstract

Background: Microalgae are efficient producers of lipid-rich biomass, making them a key component in developing a sustainable energy source, and an alternative to



fossil fuels. *Chlorella* species are of special interest because of their fast growth rate in photobioreactors. However, biological constraints still cast a significant gap between the high cost of biofuel and cheap oil, thus hampering perspective of producing CO₂-neutral biofuels. A key issue is the inefficient use of light caused by its uneven distribution in the culture that generates photoinhibition of the surface-exposed cells and darkening of the inner layers. Efficient biofuel production, thus, requires domestication, including traits which reduce optical density of cultures and enhance photoprotection.

Results: We applied two steps of mutagenesis and phenotypic selection to the microalga *Chlorella vulgaris*. First, a pale-green mutant (PG-14) was selected, with a 50% reduction of both chlorophyll content per cell and LHClI complement per PSII, with respect to WT. PG-14 showed a 30% increased photon conversion into biomass efficiency vs. WT. A second step of mutagenesis of PG-14, followed by selection for higher tolerance to Rose Bengal, led to the isolation of pale-green genotypes, exhibiting higher resistance to singlet oxygen (strains SOR). Growth in photobioreactors under high light conditions showed an enhanced biomass production of SOR strains with respect to PG-14. When compared to WT strain, biomass yield of the pale green + sor genotype was enhanced by 68%. Conclusions: Domestication of microalgae like *Chlorella vulgaris*, by optimizing both light distribution and ROS resistance, yielded an enhanced carbon assimilation rate in photobioreactor.

Simultaneous nutrition removal and high-efficiency biomass and lipid accumulation by microalgae using anaerobic digested effluent from cattle manure combined with municipal wastewater.

Biotechnol Biofuels. 2019;12:218

Authors: Luo L, Ren H, Pei X, Xie G, Xing D, Dai Y, Ren N, Liu B

Abstract

Background: Microalgae as a viable biodiesel feedstock show great potential to approach the challenges of energy shortage and environment pollution, but their economic feasibility was seriously hampered by high production cost. Thus, it is in urgent need to reduce the cost of cultivation and improve the biomass and lipid production of microalgae. In this work, anaerobic digestion effluent from cattle manure combined with municipal wastewater was used as a cost-effective medium for cultivating microalgae and expected to obtain high biomass. The pretreatment of anaerobic digested effluent containing dilution rate, sterilization and nutrient



optimization was investigated. Then, initial pH and light intensity for algal growth, lipid production and wastewater purification were optimized in this study. Results: *Scenedesmus* sp. could grow rapidly in 10% anaerobic digestion effluent from cattle manure combined with secondary sedimentation tank effluent without sterilization. Optimum nutrient additives for higher biomass were as follows: glucose 10 g/L, NaNO₃ 0.3 g/L, K₂HPO₄·3H₂O 0.01 g/L, MgSO₄·7H₂O 0.075 g/L and trace element A5 solution 1 mL/L. Biomass of 4.65 g/L and lipid productivity of 81.90 mg/L/day were achieved during 7-day cultivation accompanying over 90% of COD, NO₃⁻-N, NH₄⁺-N, and 79-88% of PO₄³⁻-P removal with optimized initial pH of 7.0 and light intensity of 5000 lx. The FAME profile in ADEC growth medium consisted in saturated (39.48%) and monounsaturated (60.52%) fatty acids with the 16- to 18-chain-length fatty acids constituting over 98% of total FAME. Conclusions: This study proves the potential of anaerobic digested effluent combined with municipal wastewater for microalgae culture, and provides an effective avenue for simultaneous microalgal lipid production and treatment of two kinds of wastewater.

Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas.

Bioresour Technol. 2019 Jun;281:10-17

Authors: Marín D, Posadas E, García D, Puyol D, Lebrero R, Muñoz R

Abstract

The potential of purple phototrophic bacteria (PPB) for the simultaneous treatment of piggery wastewater (PWW) and biogas upgrading was evaluated batchwise in gas-tight photobioreactors. PWW dilution was identified as a key parameter determining the efficiency of wastewater treatment and biomethane quality in PPB photobioreactors. Four times diluted PWW supported the most efficient total organic carbon (TOC) and total nitrogen removals (78% and 13%, respectively), with CH₄ concentrations of 90.8%. The influence of phosphorous concentration (supplementation of 50 mg L⁻¹ of P-PO₄³⁻) on PPB-based PWW treatment coupled to biogas upgrading was investigated. TOC removals of ≈60% and CH₄ concentrations of ≈90.0% were obtained regardless of phosphorus supplementation. Finally, the use of PPB and algal-bacterial consortia supported CH₄ concentrations in the upgraded biogas of 93.3% and 73.6%, respectively, which confirmed the potential PPB for biogas upgrading coupled to PWW treatment.



Use of waste carbon dioxide and pre-treated liquid digestate from biogas process for *Phaeodactylum tricornutum* cultivation in photobioreactors and open ponds.

Bioresour Technol. 2019 Nov;292:121921

Authors: Simonazzi M, Pezolesi L, Guerrini F, Vanucci S, Samorì C, Pistocchi R

Abstract

Phaeodactylum tricornutum is considered a promising source of polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA). In this study, *P. tricornutum* cultivation using waste products from anaerobic digestion (i.e. liquid digestate and CO₂) was tested and scaled-up in closed and open prototype systems. The chemical composition of algal biomass was evaluated to optimize the lipid content. Algal productivity and composition, especially in terms of PUFAs, were not modified by the use of waste CO₂. Digestate led to a lower protein (24%) content than medium (36-37%), without affecting lipid amount (about 37%). Algal and EPA productivity were nearly two-fold higher by using photobioreactors (0.075 g biomass L⁻¹ day⁻¹ and 1.62 mg EPA g⁻¹ day⁻¹) than open ponds, which are more influenced by environmental conditions. This study highlights that economic and environmental benefits could be achieved by using waste CO₂ and liquid digestate from anaerobic digestion for microalgae cultivation.

Exergy analyses of biogas production from microalgae biomass via anaerobic digestion.

Bioresour Technol. 2019 Oct;289:121709

Authors: Xiao C, Liao Q, Fu Q, Huang Y, Xia A, Shen W, Chen H, Zhu X

Abstract

Biogas production from microalgae biomass without pretreatment and with hydrothermal pretreatment involve the energy with different quality and quantity, which makes it complex to evaluate thermodynamic performance. In this paper, exergy analyses were conducted in biogas production from microalgae biomass without pretreatment, with hydrothermal pretreatment, and with solar-driven hydrothermal pretreatment. The results showed that the materials and energy flow affected exergy efficiency in biogas production from microalgae biomass. The biogas production from microalgae biomass with solar-driven hydrothermal pretreatment



achieved the highest exergy efficiency (40.85%), compared with that without pretreatment (26.2%) and with hydrothermal pretreatment (35.98%). In addition, the maximum exergy loss was caused by biogas residue, which accounted for 60.58%, 38.54%, and 35.13% of overall exergy input in biogas production from microalgae biomass without pretreatment, with hydrothermal pretreatment, and with solar-driven hydrothermal pretreatment, respectively. Exergy analyses provide important theoretical guidance to improve the performance of biogas production from microalgae biomass.

Cultivation of *Chlorella vulgaris* on unsterilized dairy-derived liquid digestate for simultaneous biofuels feedstock production and pollutant removal.

Bioresour Technol. 2019 Aug;285:121353

Authors: Zhu S, Feng S, Xu Z, Qin L, Shang C, Feng P, Wang Z, Yuan Z

Abstract

In order to assess viability of microalgae cultivation using unsterilized dairy-derived liquid digestate (DLD) for simultaneous biofuels feedstock production and contaminant removal, four DLD concentrations (25%, 50%, 75% and 100%) were used to grow *Chlorella vulgaris* in batch photobioreactors (PBRs). The 25% DLD was an ideal alternative medium in that high growth rate (0.69 d⁻¹), high lipid productivity (112.9 mg L⁻¹ d⁻¹) as well as high nutrient removal were attained. The high DLD concentration caused inhibition of microalgal growth, where COD was more inhibitive than ammonium. The presence of bacteria did not influence microalgae production because of limited growth. Microalgal growth reduced the richness and diversity of bacterial community. Furthermore, the species of Bacteroidetes, Candidatus Saccharibacteria, and Chlamydiae rather than Proteobacteria benefited microalgal-bacterial symbiosis. These findings contribute to better application of microalgal-bacterial system for large-scale microalgae cultivation as well as environmental sustainability.



Exploring the potency of integrating semi-batch operation into lipid yield performance of *Chlamydomonas* sp. Tai-03.

Bioresour Technol. 2019 Aug;285:121331

Authors: Tan CH, Show PL, Ling TC, Nagarajan D, Lee DJ, Chen WH, Chang JS

Abstract

Third generation biofuels, also known as microalgal biofuels, are promising alternatives to fossil fuels. One attractive option is microalgal biodiesel as a replacement for diesel fuel. *Chlamydomonas* sp. Tai-03 was previously optimized for maximal lipid production for biodiesel generation, achieving biomass growth and productivity of 3.48 ± 0.04 g/L and 0.43 ± 0.01 g/L/d, with lipid content and productivity of $28.6 \pm 1.41\%$ and 124.1 ± 7.57 mg/L/d. In this study, further optimization using 5% CO₂ concentration and semi-batch operation with 25% medium replacement ratio, enhanced the biomass growth and productivity to 4.15 ± 0.12 g/L and 1.23 ± 0.02 g/L/d, with lipid content and productivity of $19.4 \pm 2.0\%$ and 239.6 ± 24.8 mg/L/d. The major fatty acid methyl esters (FAMES) were palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2). These short-chain FAMES combined with high growth make *Chlamydomonas* sp. Tai-03 a suitable candidate for biodiesel synthesis.

High production of carotenoids by the green microalga *Asterarcys quadricellulare* PUMCC 5.1.1 under optimized culture conditions.

PLoS One. 2019;14(9):e0221930

Authors: Singh DP, Khattar JS, Rajput A, Chaudhary R, Singh R

Abstract

Since carotenoids are important as natural colorants, antioxidants, nutraceuticals and pharmaceuticals, the aim of the present study was to find a new good source of these pigments. We hereby report a green microalga *Asterarcys quadricellulare* PUMCC 5.1.1 as a new and good producer of carotenoids. The organism produced 35 ± 1.75 µg carotenoids mg⁻¹ dry biomass during stationary phase in control cultures. The growth and carotenoids production by the test microalga were optimized by varying nutrient growth media, pH, nitrogen and phosphate source, salinity, light quality, intensity and duration. The optimized conditions for carotenoid production were: Bold basal (BB) medium with pH 8.5, containing with 10 mM nitrate, 3.5 mM



phosphate and 0.17 mM salinity and illuminated with blue light with 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux light intensity. Cultivation of cultures in the above mentioned optimized conditions resulted in nearly 3.0 fold increase in carotenoid production compared to the control cultures grown in unmodified BB medium. Using HPTLC, four carotenoids have been identified as β -carotene, lutein, astaxanthin and canthaxanthin. Further, carotenoids were also separated and purified by flash chromatography and the amounts of purified carotenoids were determined by HPLC. The organism produced 47.0, 28.7, 15.5 and 14.0 μg β -carotene, lutein, astaxanthin and canthaxanthin mg^{-1} dry biomass, respectively, under optimized conditions. The amount of total carotenoids (118 μg mg^{-1} dry biomass) produced by *Asterarcys quadricellulare* PUMCC 5.1.1 under optimized culture conditions was significantly higher than control cultures. Thus, this microalgal strain is a promising candidate for carotenoid production at commercial level.

Nutritional Potential and Toxicological Evaluation of *Tetraselmis* sp. CTP4 Microalgal Biomass Produced in Industrial Photobioreactors.

Molecules. 2019 Sep 03;24(17):

Authors: Pereira H, Silva J, Santos T, Gangadhar KN, Raposo A, Nunes C, Coimbra MA, Gouveia L, Barreira L, Varela J

Abstract

Commercial production of microalgal biomass for food and feed is a recent worldwide trend. Although it is common to publish nutritional data for microalgae grown at the lab-scale, data about industrial strains cultivated in an industrial setting are scarce in the literature. Thus, here we present the nutritional composition and a microbiological and toxicological evaluation of *Tetraselmis* sp. CTP4 biomass, cultivated in 100-m³ photobioreactors at an industrial production facility (AlgaFarm). This microalga contained high amounts of protein (31.2 g/100 g), dietary fibres (24.6 g/100 g), digestible carbohydrates (18.1 g/100 g) and ashes (15.2 g/100 g), but low lipid content (7.04 g/100 g). The biomass displayed a balanced amount of essential amino acids, n-3 polyunsaturated fatty acids, and starch-like polysaccharides. Significant levels of chlorophyll (3.5 g/100 g), carotenoids (0.61 g/100 g), and vitamins (e.g., 79.2 mg ascorbic acid /100 g) were also found in the biomass. Conversely, pathogenic bacteria, heavy metals, cyanotoxins, mycotoxins, polycyclic aromatic hydrocarbons, and pesticides were absent. The biomass showed moderate antioxidant activity in several in vitro assays. Taken together, as the biomass produced has a balanced biochemical composition of macronutrients and (pro-



)vitamins, lacking any toxic contaminants, these results suggest that this strain can be used for nutritional applications.

Metabolomic Evaluation of Scenedesmus sp. as a Feed Ingredient Revealed Dose-Dependent Effects on Redox Balance, Intermediary and Microbial Metabolism in a Mouse Model.

Nutrients. 2019 Aug 21;11(9):

Authors: Ma Y, Zhou W, Chen P, Urriola PE, Shurson GC, Ruan R, Chen C

Abstract

Scenedesmus is a common green algae genus with high biomass productivity, and has been widely used in biofuel production and waste water management. However, the suitability and metabolic consequences of using Scenedesmus as an animal feed ingredient have not been examined in detail. In this study, the influences of consuming Scenedesmus on the metabolic status of young mice were investigated through growth performance, blood chemistry, and liquid chromatography-mass spectrometry (LC-MS)-based metabolomics. Compared to the control diet, feeding a diet containing 5% Scenedesmus improved growth performance while the diet containing 20% Scenedesmus suppressed it. Among common macronutrients-derived blood biochemicals, serum triacylglycerols and cholesterol levels were dramatically decreased by feeding the 20% Scenedesmus diet. Metabolomic analysis of liver, serum, feces, and urine samples indicated that Scenedesmus feeding greatly affected the metabolites associated with amino acid, lipid, purine, microbial metabolism, and the endogenous antioxidant system. The growth promotion effect of feeding the 5% Scenedesmus diet was associated with elevated concentrations of antioxidants, an expanded purine nucleotide cycle, and modified microbial metabolism, while the growth suppression effect of feeding the 20% Scenedesmus diet was correlated to oxidative stress, disrupted urea cycle, upregulated fatty acid oxidation, and an imbalanced lipidome. These correlations among Scenedesmus dietary inclusion rate, individual metabolite markers, and growth performance suggest the need to define the dietary inclusion rate threshold for using Scenedesmus and other microalgae supplements as feed ingredients, and also warrant further mechanistic investigations on the biological processes connecting specific constituents of Scenedesmus with the metabolic effects observed in this study.



Comparative physiological and metabolomic analyses of the hyper-accumulation of astaxanthin and lipids in *Haematococcus pluvialis* upon treatment with butylated hydroxyanisole.

Bioresour Technol. 2019 Nov;292:122002

Authors: Ding W, Li Q, Han B, Zhao Y, Geng S, Ning D, Ma T, Yu X

Abstract

The major goal of this study was to explore the functions of butylated hydroxyanisole (BHA) combined with abiotic stress on the cultivation of the microalga *Haematococcus pluvialis* for astaxanthin and lipid production. Here, the effect of BHA on astaxanthin and lipid accumulation and physiological and metabolomic profiles was investigated. These results suggested that astaxanthin content was increased by 2.17-fold compared to the control. The lipid content was enhanced by 1.22-fold. BHA treatment simultaneously reduced carbohydrates and protein and delayed the decay of chlorophyll. Furthermore, metabolomic analysis demonstrated that BHA upregulated and activated the bioprocesses involved in cellular basal metabolism and signalling systems, such as glycolysis, the TCA cycle, amino acid metabolism and the phosphatidylinositol signalling system, thus enhancing astaxanthin and lipid accumulation. Altogether, this research shows the dramatic effects of BHA on algal metabolism in the regulation of key metabolic nodes and provides novel insights into microalgal regulation and metabolism.

Impact of Cultivation Condition and Media Content on *Chlorella vulgaris* Composition.

Adv Pharm Bull. 2019 Jun;9(2):182-194

Authors: Panahi Y, Yari Khosroushahi A, Sahebkar A, Heidari HR

Abstract

Microalgae are a source material in food, pharmacy, and cosmetics industries for producing various products including high-protein nutritional supplements, synthetic pharmaceuticals, and natural colors. A promising algal source for such productions is *Chlorella vulgaris* which contains a considerable protein content. Similar to other microalgae, its desirability is minimal nutrient requirements since they are unicellular, photosynthetic, and fast-growing microorganisms. Another propitious option to be produced by *C. vulgaris* is biodiesel, since it is rich in oil too. Besides,



algal well thriving in presence of increased amount of carbon dioxide makes them a practicable alternative biofuel resource without some problems of the traditional ones. At the same time, *C. vulgaris* is also a promising source for nutraceuticals such as amino acids, vitamins, and antioxidants. This review aims to discuss the conditions need to be observed for achieving a favorable growth efficiency of the *C. vulgaris*, as well as targeted productions such as biomass, antioxidant, and biofuel. Additionally, different approaches to induce any specific production are also considered comprehensively.

Integrated evaluation of wine lees valorization to produce value-added products.

Waste Manag. 2019 Jul 15;95:70-77

Authors: Cortés A, Moreira MT, Feijoo G

Abstract

The integrated evaluation of the valorization of wine lees to produce value-added products was carried out in this study from a life-cycle perspective. The consumption of steam has been demonstrated as the main hot spot, reaching 85.7% of the impact on Fossil Depletion and 85.3% on Climate Change. Bearing in mind that four different value-added products are produced, a sensitivity analysis was carried out in order to ascertain the influence of the functional unit and the allocation method on the environmental outcomes. The performance of this system was compared to other processes that produce antioxidants from different raw materials. These processes were phycocyanin recovery from *Spirulina platensis* cyanobacterium, the production of the red antioxidant astaxanthin by microalgae and the valorization of the macroalgae *Sargassum muticum*. Wine lees valorization showed a better environmental profile throughout the entire life cycle, due to the fact that most of the operations performed are physical (solid/liquid separations, distillations, evaporations, etc.) and do not involve a large consumption of electricity or chemicals. However, there is still room for improvement, and future research should focus on optimizing the extraction of antioxidants from wine lees using two-stages aqueous systems, ultrasonic or microwave assisted extraction, in the pursuit of better performance and lower environmental impact.



Extraction of Carotenoids and Fat-Soluble Vitamins from *Tetradesmus Obliquus* Microalgae: An Optimized Approach by Using Supercritical CO₂.

Molecules. 2019 Jul 16;24(14):

Authors: Chronopoulou L, Dal Bosco C, Di Caprio F, Prosini L, Gentili A, Pagnanelli F, Palocci C

Abstract

In recent years, great attention has been focused on rapid, selective, and environmentally friendly extraction methods to recover pigments and antioxidants from microalgae. Among these, supercritical fluid extraction (SFE) represents one of the most important alternatives to traditional extraction methods carried out with the use of organic solvents. In this study, the influence of parameters such as pressure, temperature, and the addition of a polar co-solvent in the SFE yields of carotenoids and fat-soluble vitamins from *T. obliquus* biomass were evaluated. The highest extraction of alpha-tocopherol, gamma-tocopherol, and retinol was achieved at a pressure of 30 MPa and a temperature of 40 °C. It was observed that overall, the extraction yield increased considerably when a preliminary step of sample pre-treatment, based on a matrix solid phase dispersion, was applied using diatomaceous earth as a dispersing agent. The use of ethanol as a co-solvent, under certain conditions of pressure and temperature, resulted in selectively increasing the yields of only some compounds. In particular, a remarkable selectivity was observed if the extraction was carried out in the presence of ethanol at 10 MPa and 40 °C: under these conditions, it was possible to isolate menaquinone-7, a homologous of vitamin K₂, which, otherwise, cannot not recovered by using traditional extraction procedures.

First Apocarotenoids Profiling of Four Microalgae Strains.

Antioxidants (Basel). 2019 Jul 06;8(7):

Authors: Zoccali M, Giuffrida D, Salafia F, Socaciu C, Skjånes K, Dugo P, Mondello L

Abstract

Both enzymatic or oxidative carotenoids cleavages can often occur in nature and produce a wide range of bioactive apocarotenoids. Considering that no detailed information is available in the literature regarding the occurrence of apocarotenoids in microalgae species, the aim of this study was to study the extraction and characterization of apocarotenoids in four different microalgae strains:



Chlamydomonas sp. CCMP 2294, Tetraselmis chuii SAG 8-6, Nannochloropsis gaditana CCMP 526, and Chlorella sorokiniana NIVA-CHL 176. This was done for the first time using an online method coupling supercritical fluid extraction and supercritical fluid chromatography tandem mass spectrometry. A total of 29 different apocarotenoids, including various apocarotenoid fatty acid esters, were detected: apo-12'-zeaxanthinal, β -apo-12'-carotenal, apo-12'-luteinal, and apo-12'-violaxanthal. These were detected in all the investigated strains together with the two apocarotenoid esters, apo-10'-zeaxanthinal-C4:0 and apo-8'-zeaxanthinal-C8:0. The overall extraction and detection time for the apocarotenoids was less than 10 min, including apocarotenoids esters, with an overall analysis time of less than 20 min. Moreover, preliminary quantitative data showed that the β -apo-8'-carotenal content was around 0.8% and 2.4% of the parent carotenoid, in the C. sorokiniana and T. chuii strains, respectively. This methodology could be applied as a selective and efficient method for the apocarotenoids detection.

Omega-3-rich Isochrysis sp. biomass enhances brain docosahexaenoic acid levels and improves serum lipid profile and antioxidant status in Wistar rats.

J Sci Food Agric. 2019 Oct;99(13):6066-6075

Authors: Balakrishnan J, Dhavamani S, Sadasivam SG, Arumugam M, Vellaikumar S, Ramalingam J, Shanmugam K

Abstract

BACKGROUND: Isochrysis sp. is a marine microalga, rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The potential use of its biomass as an alternative source of polyunsaturated fatty acids (PUFAs) has not been studied in animal models. Male albino Wistar rats were divided into three groups and treated for 28 days. The rats were fed with (1) standard chow (control group), (2) microalgal biomass rich in EPA and DHA along with standard chow (microalga group), and (3) fish oil that contains equivalent amounts of EPA and DHA along with standard chow (fish oil group). After intervention, biochemical indices, histopathological indices, relative mRNA expression of PUFA genes, antioxidant genes, inflammatory markers, and the fatty acid profile of major tissues were studied. **RESULTS:** Animals treated with microalgal biomass showed significantly increased serum HDL levels ($P < 0.05$) and reduced oxidative stress markers with a concomitant decrease in urea and creatinine levels. Oral supplementation of microalgal biomass did not show any toxicity or damage in any major organs. The mRNA expression of



PUFA genes was significantly downregulated ($P < 0.05$) and antioxidant genes were upregulated. Furthermore, the mRNA expression of pro-inflammatory markers was significantly downregulated ($P < 0.05$) and anti-inflammatory markers were upregulated. Oral supplementation of microalgal biomass improved DHA status in brain and liver. CONCLUSION: The present study demonstrated that *Isochrysis* sp. can be used as a safe, alternative food supplement for ω -3 fatty acids. © 2019 Society of Chemical Industry.

Growth optimization, free radical scavenging and antibacterial potential of *Chlorella* sp. SRD3 extracts against clinical isolates.

J Appl Microbiol. 2019 Aug;127(2):481-494

Authors: Santhosh S, Manivannan N, Ragavendran C, Mathivanan N, Natarajan D, Hemalatha N, Dhandapani R

Abstract

AIM: The aim of present work was to explore the potential of *Chlorella* sp. SRD3 extracts for antioxidant and antibacterial activity along with the evaluation of minimum inhibitory concentration (MIC) and haemolytic activity to detect RBC cell damage.

METHODS AND RESULTS: Screening and isolation of microalgae was performed using bold basal medium under normal illuminance (at 27°C) and microscopic observation. Growth of the microalgae was optimized using a different medium and light source. The isolated microalgae incubated under fluorescent light when cultured in F/2 medium showed a highest dry biomass yield of $3.77 \pm 0.1 \text{ g l}^{-1}$, when compared to the growth under direct sunlight ($2.74 \pm 0.07 \text{ g dwt l}^{-1}$). The quantitative analysis of extracts revealed higher phenols, flavonoids and proanthocyanidins in ethyl acetate and hexane extracts followed by methanol. The antioxidant activity of extracts was tested against 1-diphenyl-2-picrylhydrazyl and ABTS radical, its reducing power assay was performed. From antibacterial activity, the two extracts showed better inhibition against Gram-negative bacteria. Also, they resulted in very low MIC values with effective activity against pathogens. In haemolytic activity, no haemolysis occurred, when the concentration ($\mu\text{g ml}^{-1}$) was below 64 for methanol and 32 for ethyl acetate extract. In addition, *Chlorella* sp. extracts were characterized by GC-MS analysis to detect the major compounds.

CONCLUSION: The polar extracts revealed satisfactory results against the clinical isolates and the compounds responsible were reflected in the GC-MS spectrum.



SIGNIFICANCE AND IMPACT OF THE STUDY: The present study revealed significant biological potentials of the green alga, *Chlorella* sp. such as antioxidant, antibacterial and hemolytic activities. Therefore, this vital source might serve as a cost-effective, alternative choice to the pharmaceutical and food industries in the near future.

Tolerance to Cyclic Desiccation in Lichen Microalgae is Related to Habitat Preference and Involves Specific Priming of the Antioxidant System.

Plant Cell Physiol. 2019 Aug 01;60(8):1880-1891

Authors: Hell AF, Gasulla F, Gonz  Lez-Hourcade MA, Del Campo EM, Centeno DC, Casano LM

Abstract

Oxidative stress is a crucial challenge for lichens exposed to cyclic desiccation and rehydration (D/R). However, strategies to overcome this potential stress are still being unraveled. Therefore, the physiological performance and antioxidant mechanisms of two lichen microalgae, *Trebouxia* sp. (TR9) and *Coccomyxa simplex* (Csol), were analyzed. TR9 was isolated from *Ramalina farinacea*, a Mediterranean fruticose epiphytic lichen adapted to xeric habitats, while Csol is the phycobiont of *Solorina saccata*, a foliaceous lichen that grows on humid rock crevices. The tolerance to desiccation of both species was tested by subjecting them to different drying conditions and to four consecutive daily cycles of D/R. Our results show that a relative humidity close to that of their habitats was crucial to maintain the photosynthetic rates. Concerning antioxidant enzymes, in general, manganese superoxide dismutases (MnSODs) were induced after desiccation and decreased after rehydration. In TR9, catalase (CAT)-A increased, and its activity was maintained after four cycles of D/R. Ascorbate peroxidase activity was detected only in Csol, while glutathione reductase increased only in TR9. Transcript levels of antioxidant enzymes indicate that most isoforms of MnSOD and FeSOD were induced by desiccation and repressed after rehydration. CAT2 gene expression was also upregulated and maintained at higher levels even after four cycles of D/R in accordance with enzymatic activities. To our knowledge, this is the first study to include the complete set of the main antioxidant enzymes in desiccation-tolerant microalgae. The results highlight the species-specific induction of the antioxidant system during cyclic D/R, suggesting a priming of oxidative defence metabolism.



Enhancement of co-production of nutritional protein and carotenoids in *Dunaliella salina* using a two-phase cultivation assisted by nitrogen level and light intensity.

Bioresour Technol. 2019 Sep;287:121398

Authors: Sui Y, Muys M, Van de Waal DB, D'Adamo S, Vermeir P, Fernandes TV, Vlaeminck SE

Abstract

Microalga *Dunaliella salina* is known for its carotenogenesis. At the same time, it can also produce high-quality protein. The optimal conditions for *D. salina* to co-produce intracellular pools of both compounds, however, are yet unknown. This study investigated a two-phase cultivation strategy to optimize combined high-quality protein and carotenoid production of *D. salina*. In phase-one, a gradient of nitrogen concentrations was tested. In phase-two, effects of nitrogen pulse and high illumination were tested. Results reveal optimized protein quantity, quality (expressed as essential amino acid index EAAI) and carotenoids content in a two-phase cultivation, where short nitrogen starvation in phase-one was followed by high illumination during phase-two. Adopting this strategy, productivities of protein, EAA and carotenoids reached 22, 7 and 3 mg/L/d, respectively, with an EAAI of 1.1. The quality of this biomass surpasses FAO/WHO standard for human nutrition, and the observed level of β -carotene presents high antioxidant pro-vitamin A activity.

Development of a Green Downstream Process for the Valorization of *Porphyridium cruentum* Biomass.

Molecules. 2019 Apr 20;24(8):

Authors: Gallego R, Martínez M, Cifuentes A, Ibáñez E, Herrero M

Abstract

As the interest in biorefinery approaches is continuously increasing, new alternatives for the downstream valorization of biomasses are sought. *Porphyridium cruentum* microalga is a good natural source for a variety of interesting bioactive compounds, including carotenoids, phycoerythrin, and sulfated polysaccharides. In the present contribution, the use of compressed fluids-based techniques is explored towards the efficient and green extraction of bioactive compounds to valorize microalgal biomass. The extraction of carotenoids was first optimized using pressurized



ethanol. The best extraction conditions involved the use of 125 °C for 20 min at 10.5 MPa. Subsequently, a sequential valorization process was devised based on the application of different steps directed towards the extraction of phycoerythrin, sulfated polysaccharides, and carotenoids, respectively. The applied pressurized conditions allowed the attainment of a good recovery of polar components without compromising the stability and extraction of carotenoids. Therefore, the proposed approach could be employed to obtain different bioactives from *P. cruentum* microalgal biomass employing green extraction processes.

Comparative study of Cu uptake and early transcriptome responses in the green microalga *Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii*.

Environ Pollut. 2019 Jul;250:331-337

Authors: Beauvais-Flück R, Slaveykova VI, Cosio C

Abstract

Microalgae are widely used as representative primary producers in ecotoxicology, while macrophytes are much less studied. Here we compared the bioavailability and cellular toxicity pathways of 2 h-exposure to 10^{-6} mol L⁻¹ Cu in the macrophyte *Elodea nuttallii* and the green microalga *Chlamydomonas reinhardtii*. Uptake rate was similar but faster in the algae than in the macrophyte, while RNA-Sequencing revealed a similar number of regulated genes. Early-regulated genes were congruent with expected adverse outcome pathways for Cu with Gene Ontology terms including gene regulation, energy metabolism, transport, cell processes, stress, antioxidant metabolism and development. However, the gene regulation level was higher in *E. nuttallii* than in *C. reinhardtii* and several categories were more represented in the macrophyte than in the microalga. Moreover, several categories including oxidative pentose phosphate pathway (OPP), nitrate metabolism and metal handling were only found for *E. nuttallii*, whereas categories such as cell motility, polyamine metabolism, mitochondrial electron transport and tricarboxylic acid cycle (TCA) were unique to *C. reinhardtii*. These differences were attributed to morphological and metabolic differences and highlighted dissimilarities between a sessile and a mobile species. Our results highlight the efficiency of transcriptomics to assess early molecular responses in biota, and the importance of studying more aquatic plants for a better understanding on the impact and fate of environmental contaminants.



Biorefinery approach and environment-friendly extraction for sustainable production of astaxanthin from marine wastes.

Crit Rev Biotechnol. 2019 Jun;39(4):469-488

Authors: Routray W, Dave D, Cheema SK, Ramakrishnan VV, Pohling J

Abstract

Microorganisms (microalgae and fungi) are currently the main sources of astaxanthin; however, this carotenoid also accumulates in crustaceans, salmonids, and birds. Seafood (derived from marine animals) processing wastes are significant sources of astaxanthin and can be employed as feed and for nutraceutical applications, where shrimp wastes are the most exploited seafood industry waste employed for astaxanthin extraction. This review discusses different sources, efficient environment-friendly extraction methods employed for astaxanthin extraction, biorefinery approaches for efficient extraction and future aspects of the application of these waste sources for commercial preparation of astaxanthin complexes. It also includes a brief overview of the advantages, disadvantages, and challenges for obtaining astaxanthin from various sources and various case scenarios integrating different biorefinery approaches.

Differential transcriptional responses of carotenoid biosynthesis genes in the marine green alga *Tetraselmis suecica* exposed to redox and non-redox active metals.

Mol Biol Rep. 2019 Feb;46(1):1167-1179

Authors: Sathasivam R, Ki JS

Abstract

The green microalga, *Tetraselmis suecica*, is commonly used in scientific, industrial, and aquacultural purposes because of its high stress tolerance and ease of culture in wide spectrums of environments. We hypothesized that carotenoids help to protect *Tetraselmis* cells from environmental stress by regulating genes in biosynthetic pathways. Here, we determined three major carotenogenic genes, phytoene synthase (PSY), phytoene desaturase (PDS), and β -lycopene cyclase (LCY-B) in *T.*



suecica, and examined the physiological parameters and gene expression responses when exposed to redox-active metals and non-redox-active metals. Phylogenetic analyses of each gene indicated that *T. suecica* clustered well with other green algae. Real-time PCR analysis showed that TsPSY, TsPDS, and TsLCY-B genes greatly responded to the redox-active metals in CuSO₄ followed by CuCl₂, but not to the non-redox-active metals. The redox-active metals strongly affected the physiology of the cells, as determined by cell counting, reactive oxygen species (ROS) imaging, and photosynthetic efficiency. This suggests that carotenoids protect the cells from oxidative damage caused by metals, thereby contributing to cell survival under various stress conditions.

PMID: 30649658 [PubMed - indexed for MEDLINE]

The effect of *Spirulina platensis* meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*).

Fish Physiol Biochem. 2019 Jun;45(3):977-986

Authors: Teimouri M, Yeganeh S, Mianji GR, Najafi M, Mahjoub S

Abstract

The study was conducted to investigate the effect of diets containing 0, 2.5, 5, 7.5, and 10% *S. platensis* meal on total antioxidant capacity (TAC) and lipid peroxidation, as well as the expression of two antioxidant enzyme genes (SOD and CAT) in rainbow trout (*Oncorhynchus mykiss*). One hundred and eighty fish, with an average initial weight of 101 ± 8 g, were cultured for 10 weeks. At the end of experiment, lipid peroxidation significantly decreased in serum of fish fed with *S. platensis* and fish fed with 5, 7.5, and 10% microalgae showed a significantly lower value compared to control and 2.5%. Inclusion of 7.5 and 10% *S. platensis* in diet also decreased lipid peroxidation in liver. TAC significantly increased with increasing level of *S. platensis*. Expression level of superoxide dismutase (SOD) and catalase (CAT) genes significantly increased in the fish liver after administration of microalgae and fish fed with 10% *S. platensis* in diet showed the highest level compared to the other treatments. The present study reveals that inclusion of 10% *S. platensis* in diet can decrease oxidative stress in rainbow trout. Therefore, *S. platensis* can be used as potential antioxidant for fish farming.



Light induces carotenoids accumulation in a heterotrophic docosahexaenoic acid producing microalga, *Crypthecodinium* sp. SUN.

Bioresour Technol. 2019 Mar;276:177-182

Authors: Sun D, Zhang Z, Zhang Y, Cheng KW, Chen F

Abstract

In the present study, the effect of various light conditions on carotenoid accumulation in a novel heterotrophic microalga, *Crypthecodinium* sp. SUN was investigated. The results showed that *C. sp. SUN* mainly produced γ -carotene and β -carotene. The total carotenoid content could reach to 12.8 mg g⁻¹ dry weight under high light intensity (100 μ mol m⁻² s⁻¹), which was >100-fold higher than that under dark condition. Besides, along with the light intensity increased, the ROS level in vivo was decreased at 48 h and 72 h. Further study showed that, light could efficiently promote the gene expression of PSY and LCYb, which explain the molecular mechanisms of carotenoids accumulation under light conditions. Meanwhile, slightly inhibited fatty acids accumulation could promote the carotenoids yield. The present work proposed that *C. sp. SUN* could be a potential carotenoid producer, and provided valuable insight for carotenoids biosynthesis.

Potential of Microalgae Carotenoids for Industrial Application.

Appl Biochem Biotechnol. 2019 Jul;188(3):602-634

Authors: Cezare-Gomes EA, Mejia-da-Silva LDC, Pérez-Mora LS, Matsudo MC, Ferreira-Camargo LS, Singh AK, de Carvalho JCM

Abstract

Microalgae cultivation, when compared to the growth of higher plants, presents many advantages such as faster growth, higher biomass productivity, and smaller land area requirement for cultivation. For this reason, microalgae are an alternative platform for carotenoid production when compared to the traditional sources. Currently, commercial microalgae production is not well developed but, fortunately, there are several studies aiming to make the large-scale production feasible by, for example, employing different cultivation systems. This review focuses on the main carotenoids from microalgae, comparing them to the traditional sources, as well as a critical analysis about different microalgae cultivation regimes that are currently available and applicable for carotenoid accumulation. Throughout this review paper,



we present relevant information about the main commercial microalgae carotenoid producers; the comparison between carotenoid content from food, vegetables, fruits, and microalgae; and the great importance and impact of these molecule applications, such as in food (nutraceuticals and functional foods), cosmetics and pharmaceutical industries, feed (colorants and additives), and healthcare area. Lastly, the different operating systems applied to these photosynthetic cultivations are critically discussed, and conclusions and perspectives are made concerning the best operating system for acquiring high cell densities and, consequently, high carotenoid accumulation.

Three-dimensional ultrastructure and hyperspectral imaging of metabolite accumulation and dynamics in Haematococcus and Chlorella.

Microscopy (Oxf). 2019 Feb 01;68(1):57-68

Authors: Ota S, Kawano S

Abstract

Phycology has developed alongside light and electron microscopy techniques. Since the 1950s, progress in the field has accelerated dramatically with the advent of electron microscopy. Transmission electron microscopes can only acquire imaging data on a 2D plane. Currently, many of the life sciences are seeking to obtain 3D images with electron microscopy for the accurate interpretation of subcellular dynamics. Three-dimensional reconstruction using serial sections is a method that can cover relatively large cells or tissues without requiring special equipment. Another challenge is monitoring secondary metabolites (such as lipids or carotenoids) in intact cells. This became feasible with hyperspectral cameras, which enable the acquisition of wide-range spectral information in living cells. Here, we review bioimaging studies on the intracellular dynamics of substances such as lipids, carotenoids and phosphorus using conventional to state-of-the-art microscopy techniques in the field of algal biorefining.

Microbial platforms to produce commercially vital carotenoids at industrial scale: an updated review of critical issues.

J Ind Microbiol Biotechnol. 2019 May;46(5):657-674



Authors: Saini RK, Keum YS

Abstract

Carotenoids are a diverse group of isoprenoid pigments that play crucial roles in plants, animals, and microorganisms, including body pigmentation, bio-communication, precursors for vitamin A, and potent antioxidant activities. With their potent antioxidant activities, carotenoids are emerging as molecules of vital importance in protecting against chronic degenerative disease, such as aging, cancer, cataract, cardiovascular, and neurodegenerative diseases. Due to countless functions in the cellular system, carotenoids are extensively used in dietary supplements, food colorants, aquaculture and poultry feed, nutraceuticals, and cosmetics. Moreover, the emerging demand for carotenoids in these vast areas has triggered their industrial-scale production. Currently, 80%-90% of carotenoids are produced synthetically by chemical synthesis. However, the demand for naturally produced carotenoids is increasing due to the health concern of synthetic counterparts. This article presents a review of the industrial production of carotenoids utilizing a number of diverse microbes, including microalgae, bacteria, and fungi, some of which have been genetically engineered to improve production titers.

The plastoquinone pool outside the thylakoid membrane serves in plant photoprotection as a reservoir of singlet oxygen scavengers.

Plant Cell Environ. 2018 10;41(10):2277-2287

Authors: Ksas B, Légeret B, Ferretti U, Chevalier A, Pospíšil P, Alric J, Havaux M

Abstract

The *Arabidopsis* *vte1* mutant is devoid of tocopherol and plastochromanol (PC-8). When exposed to excess light energy, *vte1* produced more singlet oxygen (1O_2) and suffered from extensive oxidative damage compared with the wild type. Here, we show that overexpressing the solanesyl diphosphate synthase 1 (SPS1) gene in *vte1* induced a marked accumulation of total plastoquinone (PQ-9) and rendered the *vte1* SPS1^{oex} plants tolerant to photooxidative stress, indicating that PQ-9 can replace tocopherol and PC-8 in photoprotection. High total PQ-9 levels were associated with a noticeable decrease in 1O_2 production and higher levels of Hydroxyplastoquinone (PQ-C), a 1O_2 -specific PQ-9 oxidation product. The extra PQ-9 molecules in the *vte1* SPS1^{oex} plants were stored in the plastoglobules and the chloroplast envelopes, rather than in the thylakoid membranes, whereas PQ-C was found almost exclusively in the thylakoid membranes. Upon exposure of wild-type



plants to high light, the thylakoid PQ-9 pool decreased, whereas the extrathylakoid pool remained unchanged. In *vte1* and *vte1 SPS1oex* plants, the PQ-9 losses in high light were strongly amplified, affecting also the extrathylakoid pool, and PQ-C was found in high amounts in the thylakoids. We conclude that the thylakoid PQ-9 pool acts as a 1 O₂ scavenger and is replenished from the extrathylakoid stock.

Expanding the toolbox for cryopreservation of marine and freshwater diatoms.

Sci Rep. 2018 03 09;8(1):4279

Authors: Stock W, Pinseel E, De Decker S, Seibom J, Blommaert L, Chepurnova O, Sabbe K, Vyverman W

Abstract

Diatoms constitute the most diverse group of microalgae and have long been recognised for their large biotechnological potential. In the wake of growing research interest in new model species and development of commercial applications, there is a pressing need for long-term preservation of diatom strains. While cryopreservation using dimethylsulfoxide (DMSO) as a cryoprotective agent is the preferred method for long-term strain preservation, many diatom species cannot be successfully cryopreserved using DMSO. Therefore, in this study, we studied cryopreservation success in six different diatom species, representing the major morphological and ecological diatom groups, using a range of DMSO concentrations and Plant Vitrification Solution 2 (PVS2) as an alternative cryoprotectant to DMSO. In addition, we tested whether suppressing bacterial growth by antibiotics accelerates the post-thaw recovery process. Our results show that the effects of cryoprotectant choice, its concentration and the addition of antibiotics are highly species specific. In addition, we showed that PVS2 and antibiotics are useful agents to optimize cryopreservation of algae that cannot survive the traditional cryopreservation protocol using DMSO. We conclude that a species-specific approach will remain necessary to develop protocols for diatom cryopreservation and to increase their representation in public culture collections.



Antioxidant and anti-proliferative properties of extracts from heterotrophic cultures of *Galdieria sulphuraria*.

Nat Prod Res. 2019 Jun;33(11):1659-1663

Authors: Bottone C, Camerlingo R, Miceli R, Salbitani G, Sessa G, Pirozzi G, Carfagna S

Abstract

This study explores the possibility to use the extremophilic microalga *Galdieria sulphuraria* (strain 064) as a source of natural biomolecules with beneficial and protective effects on human health. *Galdieria* was cultivated in heterotrophy conditions and cells extracts for their antioxidant and anti-proliferative properties were tested. *Galdieria* extracts showed high antioxidant power tested through ABTS assay and revealed high glutathione and phycocyanin contents. Based on Annexin-V FITC/propidium iodide and MTT analysis, algae extracts inhibited the proliferation of human adenocarcinoma A549 cells (51.2% inhibition) through the induction of apoptosis without cell cycle arrest. Besides, cytotoxicity and cytometry assays showed a positive pro-apoptotic mechanism. On these bases, we suggest that *G. sulphuraria* from heterotrophic culture, for its therapeutic potential, could be considered a good candidate for further studies with the aim to isolate bioactive anti-cancer molecules.

Functional analysis of photosynthetic pigment binding complexes in the green alga *Haematococcus pluvialis* reveals distribution of astaxanthin in Photosystems.

Sci Rep. 2017 11 24;7(1):16319

Authors: Mascia F, Girolomoni L, Alcocer MJP, Bargigia I, Perozeni F, Cazzaniga S, Cerullo G, D'Andrea C, Ballottari M

Abstract

Astaxanthin is a ketocarotenoid produced by photosynthetic microalgae. It is a pigment of high industrial interest in aquaculture, cosmetics, and nutraceuticals due to its strong antioxidant power. *Haematococcus pluvialis*, a fresh-water microalga, accumulates high levels of astaxanthin upon oxidative stress, reaching values up to 5% per dry weight. *H. pluvialis* accumulates astaxanthin in oil droplets in the cytoplasm, while the chloroplast volume is reduced. In this work, we investigate the biochemical and spectroscopic properties of the *H. pluvialis* pigment binding



complexes responsible for light harvesting and energy conversion. Our findings demonstrate that the main features of chlorophyll and carotenoid binding complexes previously reported for higher plants or *Chlamydomonas reinhardtii* are preserved under control conditions. Transition to astaxanthin rich cysts however leads to destabilization of the Photosystems. Surprisingly, astaxanthin was found to be bound to both Photosystem I and II, partially substituting β -carotene, and thus demonstrating possible astaxanthin biosynthesis in the plastids or transport from the cytoplasm to the chloroplast. Astaxanthin binding to Photosystems does not however improve their photoprotection, but rather reduces the efficiency of excitation energy transfer to the reaction centers. We thus propose that astaxanthin binding partially destabilizes Photosystem I and II.

Carotenoid profile of three microalgae/cyanobacteria species with peroxy radical scavenger capacity.

Food Res Int. 2017 10;100(Pt 1):260-266

Authors: Patias LD, Fernandes AS, Petry FC, Mercadante AZ, Jacob-Lopes E, Zepka LQ

Abstract

Carotenoids from cyanobacteria *Aphanothece microscopica* Nageli and green microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* were identified. The total carotenoid content, based on dry weight of biomass, of *A. microscopica* Nägeli, *C. vulgaris* and *S. obliquus* were 1398.88 μ g/g, 1977.02 μ g/g and 2650.70 μ g/g, respectively. A total of 23 different carotenoids were separated in all the extracts, the major ones being all-trans- β -carotene (29.3%) and all-trans-lutein (28.1%) in *Scenedesmus*; all-trans-echinenone (22.8%) and all-trans- β -carotene (17.7%) in *Chlorella*; all-trans-echinenone (28.3%) and all-trans- β -carotene (26.2%) in *Aphanothece*. The carotenoid extracts were shown to be a potent scavenger of peroxy radical, with values of 31.1 (*Chlorella*), 14.0 (*Scenedesmus*) and 7.3 (*Aphanothece*) times more potent than α -tocopherol.



The light-dependent lethal effects of 1,2-benzisothiazol-3(2H)-one and its biodegradation by freshwater microalgae.

Sci Total Environ. 2019 Jul 01;672:563-571

Authors: Wang XX, Zhang QQ, Wu YH, Dao GH, Zhang TY, Tao Y, Hu HY

Abstract

As 1,2-benzisothiazol-3(2H)-one (BIT) has been widely used in high concentrations for microbial growth control in many domestic and industrial processes, its potential eco-risk should be assessed. This study investigated the interaction between BIT and microalgae in aquatic environment as the mechanism of BIT lethal effect on microalgae was unclear and whether microalgae could efficiently remove BIT was unknown. It was found that *Chlorella vulgaris* could be killed by high concentrations of BIT, and this lethal effect was strongly enhanced when exposed to light. Inhibition of photosystem II electron transport followed by a decrease in cellular chlorophyll led to serious damage to algal photosynthesis. The excess accumulation of reactive oxygen species caused by the photosynthetic damage under light further increased the oxidative damage and promoted cell death. Under dark condition, however, the algae could tolerate higher BIT concentrations. BIT could be efficiently removed when the growth of *Scenedesmus* sp. LX1 was not completely inhibited. With an initial concentration of 4.5 mg/L, over 99% of BIT was removed during 168 hour cultivation. Microalgal biodegradation was the primary reason for this removal, and the contributions of BIT hydrolytic/photolytic degradation, microalgal growth, photosynthesis and sorption were negligibly small. These results pointed to the potential application of microalgae for efficient BIT removal from wastewater.

Influence of three microalgal-based cultivation technologies on different domestic wastewater and biogas purification in photobioreactor.

Water Environ Res. 2019 Aug;91(8):679-688

Authors: Sun S, Hu C, Gao S, Zhao Y, Xu J

Abstract

To investigate the effects of different microalgae and culture methods on the purification of domestic wastewater and biogas, *Chlorella vulgaris* and *Scenedesmus obliquus* were selected. Three different culture methods (monoculture, microalgal-



fungi cocultivation, and microalgal-activated sludge cocultivation) were used to remove nutrients from four different domestic wastewaters and remove CO₂ from raw biogas in a photobioreactor. The results show that the effluent from the centrate of pretreated urban wastewater (WW4) achieved the highest nutrient and CO₂ removal efficiency. Cocultivation of *C. vulgaris* and activated sludge achieved the highest COD, TP, and CO₂ removal efficiencies of 79.27%, 81.25%, and 60.39% with WW4, respectively. Cocultivation of *C. vulgaris* and fungi achieved the highest TN removal efficiency of 78.46% with WW4. The contents of C, N, and P in the microalgal-activated sludge symbiont after treatment exceeded 50%, 8%, and 0.8%, respectively. Highly economically efficient energy consumption was achieved with WW4 for both *C. vulgaris* and *S. obliquus*. Microalgal-activated sludge cocultivation was identified as the optimal option for the simultaneous purification of wastewater and biogas based on its high pollution and CO₂ removal efficiency. This provides a reference for the microalgal-based purification of actual domestic wastewater and raw biogas. PRACTITIONER POINTS: Nutrient and CO₂ were efficiently removed by *C. vulgaris* with activated sludge. CO₂ was removed up to 60.4% and was economically viable. Cocultivation of *C. vulgaris* and fungi could achieve the highest TN removal with WW4.

Biodiesel production through algal cultivation in urban wastewater using algal floway.

Bioresour Technol. 2019 May;280:222-228

Authors: Marella TK, Datta A, Patil MD, Dixit S, Tiwari A

Abstract

The aim of this work was to study algal floway (AFW) to treat urban wastewater and to evaluate biomass productivity, lipid contents and biodiesel production. The results indicated the seasonal average algae productivity of 34.83 g dry weight m² d⁻¹ with a nutrient removal rate of 2.52 g m² d⁻¹N and 1.25 g m² d⁻¹P while the lipid content ranged between 14 and 22% of dry cell weight with the highest lipid productivity of 9.29 g m⁻² d⁻¹ during summer. Biodiesel quality was superior during summer with high centane number and cold filter plugging point values. High eicosapentaenoic acid content was found during winter growth cycles. AFW algae community was dominated by pennate diatoms during all growing seasons. This study is one of its kinds in Indian wastewaters and it provides fundamental information for further



optimization and use of AFW to treat domestic wastewater and to produce algae biofuel feedstock.

Effect of light intensity on the characteristics of algal-bacterial granular sludge and the role of N-acyl-homoserine lactone in the granulation.

Sci Total Environ. 2019 Apr 01;659:372-383

Authors: Zhang B, Guo Y, Lens PNL, Zhang Z, Shi W, Cui F, Tay JH

Abstract

The effects of light intensity on the development of algal-bacterial granular sludge (ABGS) were investigated over a period of 12 weeks. The ABGS developed at low light intensity ($142 \pm 10 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$) exhibited excellent settling ability (SVI₃₀ of 30.9 mL/g), COD and TN removal efficiencies (97.6% and 60.4%, respectively). High light intensity ($316 \pm 12 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$) accelerated granular biomass growth (5.3 g/L) and enhanced the TP removal efficiency (83.7%). Extracellular polymeric substance (EPS) analysis revealed that low light intensity induced more large weight distribution protein production (9-12 kDa and 50-150 kDa), predominantly tryptophan and aromatic proteins. Furthermore, N-acyl-homoserine lactones (AHLs) with a side chain $\leq \text{C}_{10}$ were commonly shared in the ABGS, and the ABGS developed at low light intensity had a higher C₆- and 3OC₈-HSL content, which effectively promoted the biofilm formation. The add-back studies showed that the AHLs facilitated the regulation of EPS synthesis. Statistical analysis indicated that the AHLs content had a close correlation with the EPS production, the 50th percentile of the particle size distribution and microbial community assembly, suggesting that AHLs-mediated quorum sensing have an important ecological role in EPS expression and algal-bacterial granulation. Overall, this study describes the ABGS development at different light intensities and the mechanisms of ABGS formation treating synthetic domestic wastewater.

Combining biotechnology with circular bioeconomy: From poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen.

Environ Res. 2018 07;164:32-38



Authors: Ferreira A, Marques P, Ribeiro B, Assemany P, de Mendonça HV, Barata A, Oliveira AC, Reis A, Pinheiro HM, Gouveia L

Abstract

The ability of microalgae to grow in nutrient-rich environments and to accumulate nutrients from wastewaters (WW) makes them attractive for the sustainable and low-cost treatment of WW. The valuable biomass produced can be further used for the generation of bioenergy, animal feed, fertilizers, and biopolymers, among others. In this study, *Scenedesmus obliquus* was able to remove nutrients from different wastewaters (poultry, swine and cattle breeding, brewery and dairy industries, and urban) with removal ranges of 95-100% for nitrogen, 63-99% for phosphorus and 48-70% for chemical oxygen demand. The biomass productivity using wastewaters was higher (except for poultry) than in synthetic medium (Bristol), the highest value being obtained in brewery wastewater (1025 mg/(L.day) of freeze-dried biomass). The produced biomass contained 31-53% of proteins, 12-36% of sugars and 8-23% of lipids, regardless of the type of wastewater. The potential of the produced *Scenedesmus obliquus* biomass for the generation of BioH₂ through batch dark fermentation processes with *Enterobacter aerogenes* was evaluated. The obtained yields ranged, in mL H₂/g Volatile Solids (VS), from 50.1 for biomass from anaerobically digested cattle WW to 390 for swine WW, whereas the yield with biomass cultivated in Bristol medium was 57.6 mL H₂/gVS.

Carbon-to-nitrogen and substrate-to-inoculum ratio adjustments can improve co-digestion performance of microalgal biomass obtained from domestic wastewater treatment.

Environ Technol. 2019 Feb;40(5):614-624

Authors: Calicioglu O, Demirer GN

Abstract

This study comparatively evaluated the effect of co-substrates on anaerobic digestion (AD) and biochemical methane potential of wastewater-derived microalgal biomass, with an emphasis on carbon-to-nitrogen (C:N) and substrate-to-inoculum (S:I) ratios. A semi-continuous photobioreactor was inoculated with *Chlorella vulgaris* and the nutrient recovery potential was investigated. Derived microalgal slurry was subjected to AD in the absence and presence of co-substrates; model kitchen waste (MKW) and waste activated sludge (WAS). The results revealed that up to 99.6% of nitrogen and 91.2% of phosphorus could be removed from municipal wastewater using *C. vulgaris*. Biomethane yields were improved by co-digestion with



both MKW and WAS. The maximum biomethane yield was observed as 523 ± 25.6 ml CH₄ g VS_{added}-1, by microalgal biomass and MKW co-digestion in 50:50 ratio, at an initial chemical oxygen demand (COD) concentration of 14.0 ± 0.1 g l⁻¹, C:N ratio of 22.0, and S:I ratio of 2.2. The observed biomethane yield was 80.7% higher than that of the mono-digestion. The highest improvement achieved by 50:50 co-digestion of microalgal biomass and WAS was 15.5%, with biomethane yield of 272 ± 11.3 ml CH₄ g VS_{added}-1 at an initial COD concentration of 14.0 ± 0.1 g l⁻¹, C:N ratio of 13.0, and S:I of 2.3.

Potential of novel *Dunaliella salina* from sambhar salt lake, India, for bioremediation of hexavalent chromium from aqueous effluents: An optimized green approach.

Ecotoxicol Environ Saf. 2019 Sep 30;180:430-438

Authors: Vidyalaxmi, Kaushik G, Raza K

Abstract

The potential of *Dunaliella salina* isolated from Sambhar Salt Lake (Rajasthan, India) for biosorption of hexavalent chromium Cr(VI) in aqueous solution has been examined under optimized culture conditions. The influence of various process parameters, such as pH (6-11), incubation time (48-120 h), metal concentration (5-25 mgL⁻¹), inoculum dose (2-10% vv⁻¹), and their combination effects during Cr(VI) sorption were analyzed by means of Response Surface Methodology (RSM) based on a 3-level Box-Behnken experiment design. Microalgae showed highest chromium biosorption with 66.4% efficiency at optimum pH (8.6) and 10% (vv⁻¹) inoculum size within 120 h. The experimental data obtained were analyzed by analysis of variance (ANOVA) along with lower value of coefficient of variation (34%), indicated the well fitness of quadratic equation as proposed by response surface model. Involvement of the surface morphology of the microalgae biomass and elemental distribution was studied through Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopic (EDS), Fourier-transform infrared spectroscopy (FTIR) and X-ray Diffraction (XRD) analysis. The findings unequivocally corroborates that the novel microalgae inherits immense potential in alleviating the levels of toxic heavy metal, such as Cr(VI) from the hydrosphere at wide range of pH and metal concentrations. The present study provides a workable solution for bioremediation of hazardous heavy metals, in general, and Cr(VI) in specific from the industrial wastes like tannery effluents.



Nutrient removal from synthetic and secondary treated sewage and tannery wastewater through phycoremediation.

Environ Technol. 2019 Feb;40(6):784-792

Authors: Nagabalaji V, Sivasankari G, Srinivasan SV, Suthanthararajan R, Ravindranath E

Abstract

In this study, potential microalgae species (*Chlorella vulgaris*, *Scenedesmus dimorphus*, *Chlorococcum* sp. and *Chlamydomonas* sp.) have been studied for nutrient removal from synthetic and industrial wastewater. Batch experiments were carried out to investigate the removal performance among four chosen species at different nitrogen and phosphorus concentrations. $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were varied from 13.2 to 52.8 mg/L and 6.6 to 26.4 mg/L, respectively, by keeping N:P ratio as 2:1. In synthetic wastewater, maximum $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ removal efficiencies of 88.6% and 91.2% were obtained with *C. vulgaris* when compared to the other microalgae studied. Further studies were carried out using *C. vulgaris* in batch experiments to investigate the nutrient removal performance in secondary treated sewage, soak liquor and composite tannery effluent. Experimental results indicated that $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and chemical oxygen demand (COD) removal efficiencies were found to be 68.6%, 74%, 71.5% and 90.2%, respectively, in secondary treated sewage. Maximum removal efficiencies of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and COD in composite tannery wastewater were found to be 55%, 85.6%, 60.5% and 43.4%, respectively. In soak liquor, maximum removal efficiencies of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and COD were found to be 66.7%, 62.6%, 63.6% and 93.8%, respectively.

Microalgal Cultivation and Nutrient Removal from Digested Piggery Wastewater in a Thin-film Flat Plate Photobioreactor.

Appl Biochem Biotechnol. 2019 Apr;187(4):1488-1501

Authors: Sun ZL, Sun LQ, Chen GZ

Abstract

This work investigated the cultivation of *Chlorella vulgaris* in a thin-film flat plate



photobioreactor under outdoor conditions and using digested piggery wastewater as the culture medium. The algal cells were able to adapt quickly to the wastewater and outdoor conditions. A specific growth rate of 0.12 day⁻¹ was obtained in the exponential growth phase, which was slightly higher than that during indoor cultivation using artificial culture medium. Results showed that *Chlorella vulgaris* effectively removed TN, TP, and COD by 72.48%, 86.93%, and 85.94%. Due to the difference in culture conditions and phosphorus availability, the biomass from outdoor cultivation contained higher lipid content and more unsaturated fatty acids compared to indoor cultures, while the amino acid composition was unaffected. Results of metallic element assay indicated that the biomass cultured with wastewater conformed to the standards required for animal feed additive production. The overall cost of the biomass production in the thin-film flat plate photobioreactor (32.94 US\$/kg) was estimated to be 4.67 times lower than that of indoor cultivation (154.04 US\$/kg). Together, these results provide a basis for large-scale outdoor production of microalgae and wastewater bioremediation.

Pollutants from fish feeding recycled for microalgae production as sustainable, renewable and valuable products.

Environ Sci Pollut Res Int. 2019 Jan;26(2):1474-1486

Authors: Chan H

Abstract

Trash fish feeding of cage fish can result in marine pollution. Whole and chopped trash fish can leach pollutants such as ammonia, phosphate and protein into surrounding waters. Reduction of pollution can be achieved by recycling the wastewater generated from trash fish feeding for cultivation of microalgae. Microalgae are potent candidates for the production of renewable and sustainable products such as feed and food, health and pharmaceutical, cosmeceutical, industrial products, and biofuel. Two microalgae, *Chlorella saccharophila* and *Nannochloropsis* sp., have the potential to produce high amounts of polyunsaturated fatty acids. Furthermore, high oil content ranging from 10.7 to 13.6% is found in *Chlorella saccharophila* and up to 9.3% for *Nannochloropsis* sp. Moreover, these microalgae can also be utilized as a biofuel to give a mean calorific value of 5364 Cal/g which is higher than that of wood for *Chlorella saccharophila* and 6132 Cal/g which is equivalent to that of coal for *Nannochloropsis* sp. An alternative biofuel derived from microalgae is feasible due to the fact that they do not compete for arable land for cultivation and land crops for feed and food. This study discusses



the synergistic coupling of microalgae mass production with wastewater treatment and carbon sequestration potential for mitigation of environmental impacts and a technically viable alternative energy resource. Additionally, the de-oiled biomass byproduct after oil extraction or its whole biomass can be converted into sustainable and renewal industrial products such as bioplastic, biopaint, bioasphalt, and biobuilding components.

Case study on the effect continuous CO₂ enrichment, via biogas scrubbing, has on biomass production and wastewater treatment in a high rate algal pond.

J Environ Manage. 2019 Sep 26;251:109614

Authors: Young P, Taylor MJ, Buchanan N, Lewis J, Fallowfield HJ

Abstract

Microalgae grown in high rate algal ponds (HRAP) treating wastewater are considered a promising feed for biofuel production. Biomass productivity is often considered to be limited by carbon availability, with the addition of CO₂ being the proposed solution. Biogas from anaerobic wastewater treatment potentially provides a cheap, co-located CO₂ source. Two identical 223 m² HRAPs were constructed at Melbourne Water's Western Treatment Plant, where biogas from an anaerobic lagoon is used to generate electricity. One HRAP was fed secondary treated wastewater that had been enriched with CO₂ recovered from the biogas using industry standard biogas scrubbers, the Enriched HRAP, while the other HRAP was fed the same wastewater expect it had by passed the biogas scrubbers, the Control HRAP. The biomass production and wastewater treatment performance of the two HRAPs was compared over 12 months. The inlet to the Enriched HRAP had significantly higher free CO₂ and inorganic carbon, 175.00 ± 49.30 mg L⁻¹ and 110.00 ± 10.2 mg L⁻¹, than the inlet to the Control HRAP, 9.30 ± 7.08 mg L⁻¹ and 89.62 ± 5.12 mg L⁻¹. There were no significant differences in biomass production between the HRAPs as measured by dry matter, particulate organic carbon or nitrogen. Chlorophyll a was statistically higher in the Enriched HRAP, however, this measurement is potentially unreliable. Regarding wastewater treatment, only total nitrogen and ammonium removal differed significantly between the HRAPs, with the Control HRAP, $59.13 \pm 21.13\%$ and $76.46 \pm 32.33\%$, slightly outperforming the Enriched HRAP, $53.52 \pm 17.41\%$ and $68.76 \pm 31.17\%$. Overall, neither biomass production nor wastewater treatment was meaningfully improved by CO₂ enrichment, however, wastewater treatment was still effective in both HRAPs.



Bioprocess operation strategies with mixotrophy/photoinduction to enhance lutein production of microalga *Chlorella sorokiniana* FZU60.

Bioresour Technol. 2019 Oct;290:121798

Authors: Xie Y, Li J, Ma R, Ho SH, Shi X, Liu L, Chen J

Abstract

This study isolated and identified the lutein-enriching microalga *Chlorella sorokiniana* FZU60. Different types of media and concentrations of sodium acetate and nitrate were evaluated to improve mixotrophic growth and lutein production. Highest lutein content, production, and productivity were obtained in BG11 medium with 1 g/L acetate and 0.75 g/L nitrate. Additionally, pulse feeding with 1 g/L acetate every 48 h led to the alternation between mixotrophy and photoinduction, resulting in a lutein production of 33.6 mg/L. Most notably, excellent lutein content (9.57 mg/g) and productivity (11.57 mg/L/d) were obtained using a new multi-operation integrated strategy, and the achieved levels exceed those reported in most related studies. This work demonstrates the synergistic integration of simple and effective strategies for the enhancement of lutein production in the indigenous microalga *C. sorokiniana* FZU60 and provides new insight into the highly efficient microalgae-based lutein production.

Microalgae Brewery Wastewater Treatment: Potentials, Benefits and the Challenges.

Int J Environ Res Public Health. 2019 05 30;16(11):

Authors: Amenorfenyo DK, Huang X, Zhang Y, Zeng Q, Zhang N, Ren J, Huang Q

Abstract

Concerns about environmental safety have led to strict regulations on the discharge of final brewery effluents into water bodies. Brewery wastewater contains huge amounts of organic compounds that can cause environmental pollution. The microalgae wastewater treatment method is an emerging environmentally friendly biotechnological process. Microalgae grow well in nutrient-rich wastewater by absorbing organic nutrients and converting them into useful biomass. The harvested



biomass can be used as animal feed, as an alternative energy source for biodiesel production and as biofertilizer. This review discusses conventional and current brewery wastewater treatment methods, and the application and potential of microalgae in brewery wastewater treatment. This study also discusses the benefits as well as challenges associated with microalgae brewery and other industrial wastewater treatments.

Optimising of *Scenedesmus* sp. biomass production in chicken slaughterhouse wastewater using response surface methodology and potential utilisation as fish feeds.

Environ Sci Pollut Res Int. 2019 Apr;26(12):12089-12108

Authors: Yaakob MA, Mohamed RMSR, Al-Gheethi A, Tiew A, Kassim AHM

Abstract

Production of *Scenedesmus* sp. biomass in chicken slaughterhouse wastewater (CSWW) is a promising alternative technique for commercial culture medium due to the high nutritional content of the generated biomass to be used as fish feeds. The current work deals with optimising of biomass production in CSWW using response surface methodology (RSM) as a function of two independent variables, namely temperature (10-30 °C) and photoperiod (6-24 h). The potential application of biomass yield as fish feeds was evaluated based on carbohydrate, protein and lipid contents. The results revealed that the best operating parameters for *Scenedesmus* sp. biomass production with high contents of carbohydrates, proteins and lipids were determined at 30 °C and after 24 h. The actual and predicted values were 2.47 vs. 3.09 g, 1.44 vs. 1.27 µg/mL, 29.9 vs. 31.60% and 25.75 vs. 28.44%, respectively. Moreover, the produced biomass has a high concentration of fatty acid methyl ester (FAME) as follows: 35.91% of C15:1; 17.58% of C24:1 and 14.11% of C18:1N9T. The biomass yields have 7.98% of eicosapentaenoic acid (EPA, C20:5N3) which is more appropriate as fish feeds. The Fourier transform infrared (FTIR) analysis of biomass revealed that the main functional groups included hydroxyl (OH), aldehyde (=C-H), alkanes and acyl chain groups. Scanning electron micrograph (SEM) and energy-dispersive X-ray spectroscopic analysis (EDS) indicated that the surface morphology and element distribution in biomass produced in BBM and CSWW were varied. The findings have indicated that the biomass produced in CSWW has high potential as fish feeds.



Successful isolation of a tolerant co-flocculating microalgae towards highly efficient nitrogen removal in harsh rare earth element tailings (REEs) wastewater.

Water Res. 2019 Sep 10;166:115076

Authors: Zhang Y, Xiong Z, Yang L, Ren Z, Shao P, Shi H, Xiao X, Pavlostathis SG, Fang L, Luo X

Abstract

Acidic rare earth element tailings (REEs) wastewater with high nitrogen and low COD is the most serious and yet unsolved environmental issue in the rare earth mining industry. The effective and cheap remediation of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ from the REEs wastewater is still a huge challenge. This harsh wastewater environment results in the difficulty for common microbes and microalgae to be survived. In this work, a novel highly tolerant co-flocculating microalgae (the combination of *Scenedesmus* sp. and *Parachlorella* sp.) was successfully isolated from the rare earth mine effluent through three-year cultivation. The removal efficiency of total inorganic nitrogen (TIN) by the co-flocculating microalgae cultivation was as high as 90.9%, which is 1.9 times than the average removal efficiency (47.9%) of previously-reported microalgae species in the wastewater with COD/N ratio ranging from 0 to 1. Thus, the residual concentrations of $\text{NH}_4^+\text{-N}$ and TIN could reach the Emission Standards of Pollutants from Rare Earths Industry (GB 26451-2011). Along with the high N removal performance, other related characteristics of the co-flocculating microalgae were also revealed, such as high tolerance towards high $\text{NH}_4^+\text{-N}$ and strong acid, rapid growth and sedimentation, and simultaneous removal of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. These algae characteristics were ascribed to the specific co-flocculating community structure covered by extracellular polymeric substances.

Uptake of copper from acid mine drainage by the microalgae *Nannochloropsis oculata*.

Environ Sci Pollut Res Int. 2019 Mar;26(7):6311-6318

Authors: Martínez-Macias MDR, Correa-Murrieta MA, Villegas-Peralta Y, Dévora-Isiordia GE, Álvarez-Sánchez J, Saldivar-Cabrales J, Sánchez-Duarte RG

Abstract

The removal of heavy metals from acid mine drainage is a key factor for avoiding



damage to the environment. The microalga *Nannochloropsis oculata* was cultured in an algal medium with 0.05, 0.1, 0.15, 0.2, and 0.25 mM copper under completely defined conditions to assess its removal capacity; the effects of copper on the cell density and lipid productivity of *N. oculata* were also evaluated. The results showed that *N. oculata* was able to remove up to $99.92 \pm 0.04\%$ of the copper content in the culture medium. A total of $89.29 \pm 1.92\%$ was eliminated through metabolism, and $10.70 \pm 1.92\%$ was removed by adsorption. These findings are favorable because they indicate that a large amount of copper was extracted due to the ability of the microalga to metabolize copper ions. The cell density, growth rate, and lipid content decreased with increased concentrations of copper in the culture medium. A positive effect on the fatty acid profile was found, as the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) content improved when the copper concentration was higher than 0.1 mmol L^{-1} , which can potentiate the production of high-quality biodiesel. *N. oculata* is a good option for the treatment of acid mine drainage due to its ability to eliminate a substantial percentage of the copper present. Moreover, combining different culture systems such that heavy metals are removed to non-toxic levels in the first stage and high cell densities, which promote lipid production, is obtained in the second stage would be an advantageous strategy.

Chemical and ecotoxicological effects of the use of drinking-water treatment residuals for the remediation of soils degraded by mining activities.

Ecotoxicol Environ Saf. 2018 10;161:281-289

Authors: Alvarenga P, Ferreira C, Mourinha C, Palma P, de Varennes A

Abstract

The aim of this study was to evaluate the use of drinking-water treatment residuals (DWTR) in the amendment of a soil affected by mining activities (Aljustrel mine, Portuguese sector of the Iberian Pyrite Belt), considering the effects on its chemical, biochemical and ecotoxicological characteristics. The DWTR had neutral characteristics (pH 6.7) and an organic matter (OM) content of 575 g kg^{-1} dry matter (DM), which makes them a potential amendment for the remediation of mine degraded soils, as they may correct soil acidity and reduce the extractable metal fraction. An incubation assay, with soil and DWTR, with or without lime, was carried out to test the doses to be used in the assisted-phytostabilization experiment. Based on the results obtained, the doses of DWTR used were the equivalent to 48, 96, and 144 t DM ha^{-1} , with and without lime application (CaCO_3 11 t DM ha^{-1}). *Agrostis*



tenuis Sibth was used as the test plant. Some amendments doses were able to improve soil characteristics (pH and OM content), to decrease metal extractability by 0.01 M CaCl₂ (especially for Cu and Zn), and to allow plant growth, that did not occur in the non-amended soil. Copper, Pb and Zn concentrations in the plant material were lower than the maximum tolerable level for cattle feed, used as an indicator of risk of entry of those metals into the human food chain. The simultaneous application of DWTR (96 and 144 t ha⁻¹), with lime, allowed a reduction in the mine soil ecotoxicity, as evaluated by some lethal and sub-lethal bioassays, including luminescence inhibition of *Vibrio fischeri*, *Daphnia magna* acute immobilization test, mortality of *Thamnocephalus platyurus*, and 72-h growth inhibition of the green microalgae *Pseudokirchneriella subcapitata*. However, DWTR were unable to increase soil microbial activity, evaluated by dehydrogenase activity, an important soil-health indicator. Also, OM content and NKjeldahl, concentrations increased slightly but remained low or very low (P and K extractable concentrations were not affected). In general, the bioassays highlighted a decrease in soil ecotoxicity with the presence of lime and DWTR (144 t DM ha⁻¹). In conclusion, DWTR are recommended to amend acidic soils, with high concentrations of trace elements, but an additional application of organic or mineral fertilizers should be considered.

Effect of light/dark cycle on nitrate and phosphate removal from synthetic wastewater based on BG11 medium by *Scenedesmus* sp.

3 Biotech. 2019 Apr;9(4):150

Authors: Habibi A, Nematzadeh GA, Shariati FP, Amrei HD, Teymouri A

Abstract

In this study, microalgae growth in the synthetic wastewater and their ability to remove nutrients under different light levels was investigated. For this purpose, a comparative study was conducted on freshwater microalgae *Scenedesmus* sp. to evaluate their performance to remove nitrate and phosphate from both slaughterhouse and dairy synthetic treated wastewaters, under different light/dark cycles (12/12, 16/8 and 24/0 h), in Erlenmeyer flasks. The best light/dark cycles in Erlenmeyer flasks for nitrate and phosphate removal and growth were obtained at 24/0 h. Moreover, nitrate and phosphate removal under light conditions at 24/0 h light/dark cycles were tested in a designed open raceway pond. The maximum nitrate removal in slaughterhouse and dairy synthetic wastewater was 78% and 99.7%, and the phosphate removal was 31% and 68%, respectively. Furthermore, the highest biomass productivity in dairy and slaughterhouse synthetic wastewater



during 9 days was 0.65 g L⁻¹ and 1.5 g L⁻¹, respectively. Thus, *Scenedesmus* sp. could be potential candidates by showing their intrinsic merit, for the reduction of nitrate and phosphate residue levels from dairy and slaughterhouse synthetic wastewaters in open raceway ponds.

Microalgal biofilms: A further step over current microalgal cultivation techniques.

Sci Total Environ. 2019 Feb 15;651(Pt 2):3187-3201

Authors: Mantzorou A, Ververidis F

Abstract

The scientific community has turned its interest to microalgae lately, because of their countless applications such as wastewater treatment and pharmaceutical industry. Nevertheless, so far applied cultivation methods are still prohibitive. Ordinary cultivation techniques in which microalgae are suspended in liquid medium suffer from many bottlenecks, such as low biomass productivities, difficulty in biomass harvesting and recovery, high installation and operating cost, high water requirements etc. Although, microalgal biofilms are known to be a nuisance because of surfaces fouling, they have emerged as an innovative technology with which microalgae are developed attached to a solid surface. This technique seems to be advantageous as compared to conventional cultivation systems. Microalgal biofilm systems could resolve the problematic aspects of ordinary cultivation techniques such as low biomass productivities, water management and biomass recovery. A detailed description of this technique with respect to the parameters affecting them is reviewed in this work.

Biomass from microalgae: the potential of domestication towards sustainable biofactories.

Microb Cell Fact. 2018 Nov 10;17(1):173

Authors: Benedetti M, Vecchi V, Barera S, Dall'Osto L

Abstract

Interest in bulk biomass from microalgae, for the extraction of high-value



nutraceuticals, bio-products, animal feed and as a source of renewable fuels, is high. Advantages of microalgal vs. plant biomass production include higher yield, use of non-arable land, recovery of nutrients from wastewater, efficient carbon capture and faster development of new domesticated strains. Moreover, adaptation to a wide range of environmental conditions evolved a great genetic diversity within this polyphyletic group, making microalgae a rich source of interesting and useful metabolites. Microalgae have the potential to satisfy many global demands; however, realization of this potential requires a decrease of the current production costs. Average productivity of the most common industrial strains is far lower than maximal theoretical estimations, suggesting that identification of factors limiting biomass yield and removing bottlenecks are pivotal in domestication strategies aimed to make algal-derived bio-products profitable on the industrial scale. In particular, the light-to-biomass conversion efficiency represents a major constraint to finally fill the gap between theoretical and industrial productivity. In this respect, recent results suggest that significant yield enhancement is feasible. Full realization of this potential requires further advances in cultivation techniques, together with genetic manipulation of both algal physiology and metabolic networks, to maximize the efficiency with which solar energy is converted into biomass and bio-products. In this review, we draft the molecular events of photosynthesis which regulate the conversion of light into biomass, and discuss how these can be targeted to enhance productivity through mutagenesis, strain selection or genetic engineering. We outline major successes reached, and promising strategies to achieving significant contributions to future microalgae-based biotechnology.

Environmental building policy by the use of microalgae and decreasing of risks for Canadian oil sand sector development.

Environ Sci Pollut Res Int. 2017 Sep;24(25):20241-20253

Authors: Avagyan AB

Abstract

Environmental building recommendations aimed towards new environmental policies and management-changing decisions which as example demonstrated in consideration of the problems of Canadian oil sands operators. For the implementation of the circular economic strategy, we use an in-depth analysis of reported environmental after-consequence on all stages of the production process. The study addressed the promotion of innovative solutions for greenhouse gas emission, waste mitigation, and risk of falling in oil prices for operators of oil sands



with creating market opportunities. They include the addition of microalgae biomass in tailings ponds for improvement of the microbial balance for the water speedily cleaning, recycling, and reusing with mitigation of GHG emissions. The use of food scraps for the nutrition of microalgae will reduce greenhouse gas emission minimally, on 0.33 MtCO₂eq for Alberta and 2.63 MtCO₂eq/year for Canada. Microalgae-derived biofuel can reduce this emission for Alberta on 11.9-17.9 MtCO₂eq and for Canada on 71-106 MtCO₂eq/year, and the manufacturing of other products will adsorb up to 135.6 MtCO₂ and produce 99.2 MtO₂. The development of the Live Conserve Industry and principal step from non-efficient protection of the environment to its cultivation in a large scale with mitigation of GHG emission and waste as well as generating of O₂ and value-added products by the use of microalgae opens an important shift towards a new design and building of a biological system.

Chlorella vulgaris mixotrophic growth enhanced biomass productivity and reduced toxicity from agro-industrial by-products.

Chemosphere. 2018 Aug;204:344-350

Authors: Melo RG, Andrade AF, Bezerra RP, Correia DS, Souza VC, Brasileiro-Vidal AC, Viana Marques DA, Porto ALF

Abstract

Algal wastewater remediation has become attractive for a couple of years now, however the effectiveness of genetic toxicity reducing of some by-products through microalgae are still not well reported. This study aimed to evaluate the growth, nutrients and toxicity removal of *Chlorella vulgaris* cultivated under autotrophic and mixotrophic conditions in three agro-industrial by-products. Mixotrophic culture using corn steep liquor showed higher cell concentration, specific growth rate, maximum cell productivity and biomass protein content when compared to cheese whey and vinasse. Nutrient removal results showed that *C. vulgaris* was able to completely remove corn steep liquor nutrients, while in cheese whey and vinasse culture this removal was not as efficient, observing remaining COD. This work evaluated for the first time the corn steep liquor and cheese whey genetic toxicity through *Allium cepa* seeds assay. These results demonstrate that corn steep liquor toxicity was totally eliminated by *C. vulgaris* cultivation, and cheese whey and vinasse toxicity were minimized. This study proves that the mixotrophic cultivation of *C. vulgaris* can increase cellular productivity, as well as it is a suitable and economic alternative to remove the toxicity from agroindustrial by-products.



Fed-batch cultivation of *Arthrospira* and *Chlorella* in ammonia-rich wastewater: Optimization of nutrient removal and biomass production.

Bioresour Technol. 2015 Oct;193:35-41

Authors: Markou G

Abstract

In the present work the cyanobacterium *Arthrospira platensis* and the microalga *Chlorella vulgaris* were fed-batch cultivated in ammonia-rich wastewater derived from the anaerobic digestion of poultry litter. Aim of the study was to maximize the biomass production along with the nutrient removal aiming to wastewater treatment. Ammonia and phosphorus removals were very high (>95%) for all cultures investigated. Both microorganisms were able to remove volatile fatty acids to an extent of >90%, indicating that they were capable of mixotrophic growth. Chemical oxygen demand and proteins were also removed in various degrees. In contrast, in all cultures carbohydrate concentration was increased. The biochemical composition of the microorganisms varied greatly and was influenced by the indicate that the nutrient availability. *A. platensis* accumulated carbohydrates ($\approx 40\%$), while *C. vulgaris* accumulated lipids ($\approx 50\%$), rendering them interesting for biofuel production.

Microalgal system for treatment of effluent from poultry litter anaerobic digestion.

Bioresour Technol. 2011 Dec;102(23):10841-8

Authors: Singh M, Reynolds DL, Das KC

Abstract

The potential of mixotrophic microalgae to utilize poultry litter anaerobic digester (AD) effluent (PLDE) as nutritional growth medium was evaluated. Three algal strains viz. *Chlorella minutissima*, *Chlorella sorokiniana* and *Scenedesmus bijuga* and their consortium showed significant biomass productivity in 6% (v/v) concentration of PLDE in deionized water. Multiple booster dosage of PLDE supported better growth



relative to a single dose PLDE. The maximum biomass productivity of 76 mg L⁻¹ d⁻¹ was recorded. The biomass was rich in protein (39% w/w) and carbohydrates (22%) while lipids (<10%) were low, making it most suitable as an animal feed supplement. The mixotrophic algae showed sustainable growth against variations in PLDE composition in different AD batches, thus proving to be a suitable candidate for large scale wastewater treatment with concomitant production of renewable biomass feedstock for animal feed and bioenergy applications.

Bioremediation of domestic and industrial wastewaters integrated with enhanced biodiesel production using novel oleaginous microalgae.

Environ Sci Pollut Res Int. 2016 Oct;23(20):20997-21007

Authors: Arora N, Patel A, Sartaj K, Pruthi PA, Pruthi V

Abstract

The study illustrates the synergistic potential of novel microalgal, *Chlamydomonas debaryana* IITRIND3, for phycoremediation of domestic, sewage, paper mill and dairy wastewaters and then subsequent utilisation of its biomass for biodiesel production. Among these wastewaters, maximum lipid productivity (87.5 ± 2.3 mg L⁻¹ day⁻¹) was obtained in dairy wastewater with removal efficiency of total nitrogen, total phosphorous, chemical oxygen demand and total organic carbon to be 87.56, 82.17, 78.57 and 85.97 %, respectively. Metal ions such as sodium, calcium, potassium and magnesium were also removed efficiently from the wastewaters tested. Pigment analysis revealed loss of chlorophyll a while increase in carotenoid content in algal cells cultivated in different wastewaters. Biochemical data of microalgae grown in different wastewaters showed reduction in protein content with an increase in carbohydrate and lipid contents. The major fatty acids in algal cells grown in dairy wastewater were C14:0, C16:0, C16:1, C18:0, C18:2 and C18:3. The physical properties of biodiesel derived from microalgae grown in dairy wastewater were in compliance with the ASTM D6751 and EN 14214 fuel standards and were comparable to plant oil methyl esters.

Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production.



Environ Sci Pollut Res Int. 2016 May;23(9):8379-87

Authors: Qin L, Wang Z, Sun Y, Shu Q, Feng P, Zhu L, Xu J, Yuan Z

Abstract

The potential of microalgae consortia used in dairy wastewater treatment combined with microalgae biodiesel feedstock production was evaluated by comparing the nutrient removal of dairy wastewater, the growth of cells, and the lipid content and composition of biomass between monoalgae and microalgae consortia cultivation system. Our results showed that higher chemical oxygen demand (COD) removal (maximum, 57.01-62.86 %) and total phosphorus (TP) removal (maximum, 91.16-95.96 %) were achieved in almost microalgae consortia cultivation system than those in *Chlorella* sp. monoalgae cultivation system (maximum, 44.76 and 86.74 %, respectively). In addition, microalgae consortia cultivation except the mixture of *Chlorella* sp. and *Scenedesmus* spp. reached higher biomass concentration (5.11-5.41 g L⁻¹), biomass productivity (730.4-773.2 mg L⁻¹ day⁻¹), and lipid productivity (143.7-150.6 mg L⁻¹ day⁻¹) than those of monoalgae cultivation (4.72 g L⁻¹, 674.3, and 142.2 mg L⁻¹ day⁻¹, respectively) on the seventh day. Furthermore, the fatty acid methyl ester (FAME) profiles indicated the lipids produced from microalgae consortia cultivation system were more suitable for biodiesel production. The microalgae consortia display superiority in dairy wastewater treatment and the getting feedstock for biodiesel production.

Cultivation of *Chlorella* sp. using raw dairy wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures.

Bioresour Technol. 2015 Sep;192:382-8

Authors: Lu W, Wang Z, Wang X, Yuan Z

Abstract

The biomass productivity and nutrient removal capacity of simultaneous *Chlorella* sp. cultivation for biodiesel production and nutrient removal in raw dairy wastewater (RDW) in indoor bench-scale and outdoor pilot-scale photobioreactors were compared. Results from the current work show that maximum biomass productivity in indoor bench-scale cultures can reach 260 mg L⁻¹ day⁻¹, compared to that of 110 mg L⁻¹ day⁻¹ in outdoor pilot-scale cultures. Maximum chemical oxygen demand (COD), total nitrogen (TN), and total phosphorous (TP) removal rate



obtained in indoor conditions was 88.38, 38.34, and 2.03 mg L⁻¹ day⁻¹, respectively, this compared to 41.31, 6.58, and 2.74 mg L⁻¹ day⁻¹, respectively, for outdoor conditions. Finally, dominant fatty acids determined to be C16/C18 in outdoor pilot-scale cultures indicated great potential for scale up of *Chlorella* sp. cultivation in RDW for high quality biodiesel production coupling with RDW treatment.

Three stage cultivation process of facultative strain of *Chlorella sorokiniana* for treating dairy farm effluent and lipid enhancement.

Water Res. 2015 Sep 01;80:346-56

Authors: Hena S, Fatihah N, Tabassum S, Ismail N

Abstract

Reserve lipids of microalgae are promising for biodiesel production. However, economically feasible and sustainable energy production from microalgae requires optimization of cultivation conditions for both biomass yield and lipid production of microalgae. Biomass yield and lipid production in microalgae are a contradictory problem because required conditions for both targets are different. Simultaneously, the mass cultivation of microalgae for biofuel production also depends extremely on the performance of the microalgae strains used. In this study a green unicellular microalgae *Chlorella sorokiniana* (DS6) isolated from the holding tanks of farm wastewater treatment plant using multi-step screening and acclimation procedures was found high-lipid producing facultative heterotrophic microalgae strain capable of growing on dairy farm effluent (DFE) for biodiesel feedstock and wastewater treatment. Morphological features and the phylogenetic analysis for the 18S rRNA identified the isolated strains. A novel three stage cultivation process of facultative strain of *C. sorokiniana* was examined for lipid production.

Cultivation of microalgae in dairy farm wastewater without sterilization.

Int J Phytoremediation. 2015;17(1-6):222-7

Authors: Ding J, Zhao F, Cao Y, Xing L, Liu W, Mei S, Li S



Abstract

The present study investigated the feasibility of cultivating microalgae in dairy farm wastewater. The growth of microalgae and the removal rate of the nutrient from the wastewater were examined. The wastewater was diluted 20, 10 and 5 times before applied to cultivate microalgae. A 5 dilution yielded 0.86 g/L dry weight in 6 days with a relative growth rate of 0.28 d⁻¹, the 10×dilution gave 0.74 g/L and a relative growth rate of 0.26 d⁻¹ while the 20×dilution 0.59 g/L and a relative growth rate 0.23 d⁻¹. The nutrients in the wastewater could be removed effectively in different diluted dairy wastewater. The greatest dilution (20×) showed the removal rates: ammonia, 99.26%; P, 89.92%; COD, 84.18%. A 10×dilution removal% was: ammonia 93; P 91 and COD 88. The 5× dilution removal% was: ammonia 83; P 92; COD 90.

Microalgal biomass and lipid production in mixed municipal, dairy, pulp and paper wastewater together with added flue gases.

Bioresour Technol. 2014 Oct;169:27-32

Authors: Gentili FG

Abstract

The aim of the study was to grow microalgae on mixed municipal and industrial wastewater to simultaneously treat the wastewater and produce biomass and lipids. All algal strains grew in all wastewater mixtures; however, *Selenastrum minutum* had the highest biomass and lipids yields, up to 37% of the dry matter. Nitrogen and phosphorus removal were high and followed a similar trend in all three strains. Ammonium was reduced from 96% to 99%; this reduction was due to algal growth and not to stripping to the atmosphere, as confirmed by the amount of nitrogen in the dry algal biomass. Phosphate was reduced from 91% to 99%. In all strains used the lipid content was negatively correlated to the nitrogen concentration in the algal biomass. Mixtures of pulp and paper wastewater with municipal and dairy wastewater have great potential to grow algae for biomass and lipid production together with effective wastewater treatment.



Dairy wastewater treatment using an activated sludge-microalgae system at different light intensities.

Water Sci Technol. 2014;69(8):1598-605

Authors: Tricolici O, Bumbac C, Patroescu V, Postolache C

Abstract

A microalgae-bacteria system was used for dairy industry wastewater treatment in sequenced batch mode in a photobioreactor. The research investigated the influence of two light intensities: 360 and 820 $\mu\text{mol m}^{-2}\text{s}^{-1}$ on treatment performances, microalgal cell recovery and dynamics of the protozoan community. Results showed that the light intensity of 360 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was found to be insufficient to support photosynthetic activity after the increase of bacterial biomass leading to the decrease of organic matter and ammonium removal efficiencies from 95 to 78% and 95 to 41%, respectively. Maximum microalgal cells recovery was about 63%. Continuous modification in the protozoan community was also noticed during this test. Increasing the light intensity to 820 $\mu\text{mol m}^{-2}\text{s}^{-1}$ led to better microalgal cells recovery (up to 88%) and improved treatment performances. However, the decrease of protozoan richness to small flagellates and free-swimming ciliates was noticed. Moreover, the developed protozoan trophic network was found to be different from that identified in the conventional activated sludge system. The study emphasized that high increase of bacterial biomass promoted in nutrient- and organic matter-rich wastewater can strongly affect the treatment performances as a result of the shadow effect produced on the photoautotrophic microalgae aggregates.

Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load.

Bioresour Technol. 2014 Aug;165:295-301

Authors: Ummalyima SB, Sukumaran RK

Abstract

Dairy effluent (DE) was evaluated for cultivation of the oleaginous micro alga *Chlorococcum* sp. RAP13 under mixotrophic and heterotrophic modes. The alga grew better and accumulated more lipids under heterotrophic cultivation. Supplementation of biodiesel industry waste glycerol (BDWG) to DE enhanced the



biomass production as well as lipid accumulation. While the biomass yield was 0.8g/L for mixotrophic cultivation, it was 1.48g/L and 1.94g/L respectively when cultivated with 4% or 6% BDWG. The cells accumulated 31% lipid when grown in mixotrophic mode, and heterotrophic cultivation with 4% or 6% BDWG resulted in a lipid accumulation of 39% and 42% respectively. Saturated fatty acids production was elevated in the DE, and the major fatty acid components of the algal oil were palmitic (16:0), oleic (18:1), stearic (18:0), linoleic (18:2) and linolenic (18:3) acids. DE quality improved with reduction in COD and BOD after algal cultivation.

Cultivation of *Chlorella vulgaris* in dairy wastewater pretreated by UV irradiation and sodium hypochlorite.

Appl Biochem Biotechnol. 2014 Jan;172(2):1121-30

Authors: Qin L, Shu Q, Wang Z, Shang C, Zhu S, Xu J, Li R, Zhu L, Yuan Z

Abstract

There is potential in the utilization of microalgae for the purification of wastewater as well as recycling the resource in the wastewater to produce biodiesel. The large-scale cultivation of microalgae requires pretreatment of the wastewater to eliminate bacteria and protozoa. This procedure is costly and complex. In this study, two methods of pretreatment, UV irradiation, and sodium hypochlorite (NaClO), in various doses and concentrations, were tested in the dairy wastewater. Combining the efficiency of biodiesel production, we proposed to treat the dairy wastewater with NaClO in the concentration of 30 ppm. In this condition, The highest biomass productivity and lipid productivity of *Chlorella vulgaris* reached 0.450 g L⁽⁻¹⁾ day⁽⁻¹⁾ and 51 mg L⁽⁻¹⁾ day⁽⁻¹⁾ after a 4-day cultivation in the dairy wastewater, respectively.

Production of biodiesel from microalgae *Chlamydomonas polypyrenoideum* grown on dairy industry wastewater.

Bioresour Technol. 2013 Sep;144:499-503

Authors: Kothari R, Prasad R, Kumar V, Singh DP



Abstract

This study involves a process of phyco-remediation of dairy industry wastewater by algal strain *Chlamydomonas polypyrenoideum*. The results of selected algal strain indicated that dairy industry wastewater was good nutrient supplement for algal growth in comparable with BG-11 growth medium. Alga grown on dairy industry wastewater reduced the pollution load of nitrate (90%), nitrite (74%), phosphate (70%), chloride (61%), fluoride (58%), and ammonia (90%) on 10th day of its growth as compared to that of uninoculated wastewater. The lipid content of algal biomass grown on dairy wastewater on 10th day (1.6g) and 15th day (1.2 g) of batch experiment was found to be higher than the lipid content of algal biomass grown in BG-11 growth medium on 10th day (1.27 g) and 15th day (1.0 g) of batch experiment. The results on FTIR analysis of the extracted bio-oil through transesterification reaction was comparable with bio-oil obtained from other sources.

Coupling process study of lipid production and mercury bioremediation by biomimetic mineralized microalgae.

Bioresour Technol. 2017 Nov;243:628-633

Authors: Peng Y, Deng A, Gong X, Li X, Zhang Y

Abstract

Considering the high concentration of mercury in industrial wastewater, such as coal-fired power plants and gold mining wastewater, this research study investigated the coupling process of lipid production and mercury bioremediation using microalgae cells. *Chlorella vulgaris* modified by biomimetic mineralization. The cultivation was divided in two stages: a natural cultivation for 7days and 5days of Hg²⁺ addition (10-100µg/L) for cultivation at different pH values (4-7) after inoculation. Next, the harvested cells were eluted, and lipid was extracted. The fluorescein diacetate (FDA) dye tests demonstrated that the mineralized layer enhanced the biological activity of microalgae cells in Hg²⁺ contaminated media. Hg distribution tests showed that the Hg removal capacity of modified cells was increased from 62.85% to 94.74%, and 88.72% of eluted Hg²⁺ concentration was observed in modified cells compared to 48.42% of raw cells, implying that more mercury was transferred from lipid and residuals into elutable forms.



Continuous removal of zinc from wastewater and mine dump leachate by a microalgal biofilm PSBR.

J Hazard Mater. 2015 Oct 30;297:112-8

Authors: Li T, Lin G, Podola B, Melkonian M

Abstract

Bio-removal of heavy metals from wastewater by microalgae has been investigated for decades. However, technical and economical limitations of cultivation systems for microalgae still impair progress toward application. Recently, a novel type of bioreactor for (immobilized) biofilm cultivation, the Porous Substrate Bioreactor (PSBR), has been shown to optimize biomass feedstock production and harvest, offering novel possibilities for application in the treatment of wastewater. We used two types of laboratory-scale Twin-Layer PSBRs to remove zinc (2-3 mg Zn L⁻¹) from synthetic wastewater and real mine dump leachate in a continuous and batch process. The selection and use of a biofilm of a Zn-resistant strain of the green alga *Stichococcus bacillaris* (EC50 of 28.9 mg Zn L⁻¹ based on Pulse-amplitude modulated (PAM) chlorophyll fluorescence analysis) led to a high zinc absorption capacity of 15-19 mg Zn g⁻¹ algal dry matter. The removal capacity for zinc correlated positively with biomass production and was thus, light dependent. Bio-removal properties observed here combined with biomass productivities of PSBR systems compare favorably with other algal-based bio-sorption technologies.

Heavy metal removal from acid mine drainage by calcined eggshell and microalgae hybrid system.

Environ Sci Pollut Res Int. 2015 Sep;22(17):13404-11

Authors: Choi HJ, Lee SM

Abstract

This study investigates the use of calcined eggshells and microalgae for the removal of heavy metals from acid mine drainage (AMD) and the simultaneous enhancement of biomass productivity. The experiment was conducted over a period of 6 days in a hybrid system containing calcined eggshells and the microalgae *Chlorella vulgaris*. The results show that the biomass productivity increased to ~8.04 times its initial concentration of 0.367 g/L as measured by an optical panel photobioreactor (OPPBR) and had a light transmittance of 95 % at a depth of 305 mm. On the other hand, the



simultaneous percent removal of Fe, Cu, Zn, Mn, As, and Cd from the AMD effluent was found to be 99.47 to 100 %. These results indicate that the hybrid system with calcined eggshells and microalgae was highly effective for heavy metal removal in the AMD.

Profiling microbial communities in manganese remediation systems treating coal mine drainage.

Appl Environ Microbiol. 2015 Mar;81(6):2189-98

Authors: Chaput DL, Hansel CM, Burgos WD, Santelli CM

Abstract

Water discharging from abandoned coal mines can contain extremely high manganese levels. Removing this metal is an ongoing challenge. Passive Mn(II) removal beds (MRBs) contain microorganisms that oxidize soluble Mn(II) to insoluble Mn(III/IV) minerals, but system performance is unpredictable. Using amplicon pyrosequencing, we profiled the bacterial, fungal, algal, and archaeal communities in four MRBs, performing at different levels, in Pennsylvania to determine whether they differed among MRBs and from surrounding soil and to establish the relative abundance of known Mn(II) oxidizers. Archaea were not detected; PCRs with archaeal primers returned only nontarget bacterial sequences. Fungal taxonomic profiles differed starkly between sites that remove the majority of influent Mn and those that do not, with the former being dominated by Ascomycota (mostly Dothideomycetes) and the latter by Basidiomycota (almost entirely Agaricomycetes). Taxonomic profiles for the other groups did not differ significantly between MRBs, but operational taxonomic unit-based analyses showed significant clustering by MRB with all three groups ($P < 0.05$). Soil samples clustered separately from MRBs in all groups except fungi, whose soil samples clustered loosely with their respective MRB. Known Mn(II) oxidizers accounted for a minor proportion of bacterial sequences (up to 0.20%) but a greater proportion of fungal sequences (up to 14.78%). MRB communities are more diverse than previously thought, and more organisms may be capable of Mn(II) oxidation than are currently known.



Isolation of novel microalgae from acid mine drainage and its potential application for biodiesel production.

Appl Biochem Biotechnol. 2014 Aug;173(8):2054-64

Authors: Yun HS, Lee H, Park YT, Ji MK, Kabra AN, Jeon C, Jeon BH, Choi J

Abstract

Microalgae were selected and isolated from acid mine drainage in order to find microalgae species which could be cultivated in low pH condition. In the present investigation, 30 microalgae were isolated from ten locations of acid mine drainage in South Korea. Four microalgae were selected based on their growth rate, morphology, and identified as strains of KGE1, KGE3, KGE4, and KGE7. The dry biomass of microalgae species ranged between 1 and 2 g L⁻¹ after 21 days of cultivation. The growth kinetics of microalgae was well described by logistic growth model. Among these, KGE7 has the highest biomass production (2.05 ± 0.35 g L⁻¹), lipid productivity (0.82 ± 0.14 g L⁻¹), and C16-C18 fatty acid contents (97.6 %). These results suggest that *Scenedesmus* sp. KGE 7 can be utilized for biodiesel production based on its high biomass and lipid productivity.

Effect of mine wastewater on nutrient removal and lipid production by a green microalga *Micratinium reisseri* from concentrated municipal wastewater.

Bioresour Technol. 2014 Apr;157:84-90

Authors: Ji MK, Kabra AN, Salama el-S, Roh HS, Kim JR, Lee DS, Jeon BH

Abstract

Effect of mine wastewater on the nutrient removal efficiency of a green microalga *Micratinium reisseri* from concentrated municipal wastewater (CMW) with simultaneous lipid production was investigated. Different dilution ratios (1-10%) of CMW either with mine wastewater (MWF) or mine wastewater without Fe (MWO) were used. *M. reisseri* showed the highest growth (0.8g L⁻¹) and nutrient uptake (35.9mg TNL⁻¹ and 5.4mg TPL⁻¹) at 3% MWF ([Fe]_{tot}=6.7mg L⁻¹), and the highest lipid productivity (10.4mg L⁻¹ day⁻¹) at 5% MWF ([Fe]_{tot}=11.2mg L⁻¹) after 15 days. CMW supported the algal autoflocculation due to formation of phosphate, calcium and magnesium precipitates at a high suspension pH. Fatty acid methyl ester analysis revealed that the microalgal lipids possessed 79-82% of C16/C18 fatty acids.



Application of mine wastewater improved the nutrient removal efficiency, growth and lipid productivity of *M. reisseri* cultivated in CMW.

The effect of CO₂ on algal growth in industrial waste water for bioenergy and bioremediation applications.

PLoS One. 2013;8(11):e81631

Authors: Roberts DA, de Nys R, Paul NA

Abstract

The energy, mining and mineral processing industries are point sources of metal-contaminated waste water and carbon dioxide (CO₂). Freshwater macroalgae from the genus *Oedogonium* can be grown in metal-contaminated waste water to generate biomass for bioenergy applications and concomitantly bioremediate metals. However, interactions between CO₂ addition and algal growth, which can affect bioremediation, remain untested. The addition of CO₂ to algal cultures in the Ash Dam Water (ADW) from a coal-fired power station increased the biomass productivity of *Oedogonium* sp. from 6.8 g dry weight (DW) m⁽⁻²⁾ d⁽⁻¹⁾ to a maximum of 22.5 g DW m⁽⁻²⁾ d⁽⁻¹⁾. The greater productivity increased the rate of bioremediation of most elements. However, over time carbon-amended cultures experienced a decline in productivity. Possible explanations include metal toxicity at low pH or essential trace element limitation as a result of competition between toxic and essential trace elements for uptake into algae. Higher productivity increased bioremediation rate and yielded more biomass for bioenergy applications, making maintenance of maximum productivity the central aim of the integrated culture model. To do so it will be necessary to resolve the mechanisms responsible for declining yields over time in carbon-amended cultures. Regardless, our data demonstrate that freshwater macroalgae are ideal candidates for bioremediation of metal-contaminated waste streams. Algal culture delivered significant improvement in ADW quality, reducing 5 elements that were initially in excess of water quality criteria (Al, As, Cd, Ni and Zn) to meet guidelines within two to four weeks.



Rapid adaptation of microalgae to bodies of water with extreme pollution from uranium mining: an explanation of how mesophilic organisms can rapidly colonise extremely toxic environments.

Aquat Toxicol. 2013 Nov 15;144-145:116-23

Authors: García-Balboa C, Baselga-Cervera B, García-Sánchez A, Igual JM, Lopez-Rodas V, Costas E

Abstract

Extreme environments may support communities of microalgae living at the limits of their tolerance. It is usually assumed that these extreme environments are inhabited by extremophile species. However, global anthropogenic environmental changes are generating new extreme environments, such as mining-effluent pools of residual waters from uranium mining with high U levels, acidity and radioactivity in Salamanca (Spain). Certain microalgal species have rapidly adapted to these extreme waters (uranium mining in this area began in 1960). Experiments have demonstrated that physiological acclimatisation would be unable to achieve adaptation. In contrast, rapid genetic adaptation was observed in waters ostensibly lethal to microalgae by means of rare spontaneous mutations that occurred prior to the exposure to effluent waters from uranium mining. However, adaptation to the most extreme conditions was only possible after recombination through sexual mating because adaptation requires more than one mutation. Microalgae living in extreme environments could be the descendants of pre-selective mutants that confer significant adaptive value to extreme contamination. These "lucky mutants" could allow for the evolutionary rescue of populations faced with rapid environmental change.

Removal of metal from acid mine drainage using a hybrid system including a pipes inserted microalgae reactor.

Bioresour Technol. 2013 Dec;150:242-8

Authors: Park YT, Lee H, Yun HS, Song KG, Yeom SH, Choi J

Abstract

In this study, the microalgae culture system to combined active treatment system and pipe inserted microalgae reactor (PIMR) was investigated. After pretreated AMD in active treatment system, the effluent load to PIMR in order to *Nephroselmis* sp.



KGE 8 culture. In experiment, effect of iron on growth and lipid accumulation in microalgae were inspected. The 2nd pretreatment effluent was economic feasibility of microalgae culture and lipid accumulation. The growth kinetics of the microalgae are modeled using logistic growth model and the model is primarily parameterized from data obtained through an experimental study where PIMR were dosed with BBM, BBM added 10 mg L⁻¹ iron and 2nd pretreatment effluent. Moreover, the continuous of microalgae culture in PIMR can be available. Overall, this study indicated that the use of pretreated AMD is a viable method for culture microalgae and lipid accumulation.

Algae-based biofilm productivity utilizing dairy wastewater: effects of temperature and organic carbon concentration.

J Biol Eng. 2016;10:18

Authors: Fica ZT, Sims RC

Abstract

BACKGROUND: Biofilm-based microalgal growth was determined as functions of organic chemical loading and water temperature utilizing dairy wastewater from a full-scale dairy farm. The dairy industry is a significant source of wastewater worldwide that could provide an inexpensive and nutrient rich feedstock for the cultivation of algae biomass for use in downstream processing of animal feed and aquaculture applications. Algal biomass was cultivated using a Rotating Algal Biofilm Reactor (RABR) system. The RABR is a biofilm-based technology that has been designed and used to remediate municipal wastewater and was applied to treat dairy wastewater through nutrient uptake, and simultaneously provide biomass for the production of renewable bioproducts.

RESULTS: Aerial algal biofilm growth rates in dairy wastewater at 7 and 27 °C temperatures were shown to be 4.55 ± 0.17 g/m²-day and 7.57 ± 1.12 g/m²-day ash free dry weight (AFDW), respectively. Analysis of Variance (ANOVA) calculations indicated that both an increase in temperature of the wastewater and an increase in the level of organic carbon, from 300 to 1200 mg L⁻¹, contributed significantly to an increase in the rate of biomass growth in the system. However, ANOVA results indicated that the interaction of temperature and organic carbon content was not significantly related to the biofilm-based growth rate.

CONCLUSION: A microalgae-based biofilm reactor was successfully used to treat turbid dairy wastewater. Temperature and organic carbon concentration had a



statistically significant effect on algae-based biofilm productivity and treatment of dairy wastewater. The relationships between temperature, TOC, and productivity developed in this study may be used in the design and assessment of wastewater remediation systems and biomass production systems utilizing algae-based biofilm reactors for treating dairy wastes.

Economic feasibility of microalgal bacterial floc production for wastewater treatment and biomass valorization: A detailed up-to-date analysis of up-scaled pilot results.

Bioresour Technol. 2017 Jan;224:118-129

Authors: Vulsteke E, Van Den Hende S, Bourez L, Capoen H, Rousseau DPL, Albrecht J

Abstract

The economic potential of outdoor microalgal bacterial floc (MaB-floc) raceway ponds as wastewater treatment technology and bioresource of biomass for fertilizer, shrimp feed, phycobiliproteins and biogas in Northwest Europe is assessed. This assessment is based on cost data provided by industry experts, on experimental data obtained from pilot-scale outdoor MaB-floc ponds treating aquaculture and food-industry effluents, and from different biomass valorization tests. MaB-floc ponds exhibit a cost-performance of EUR 0.25-0.50m⁻³ wastewater which is similar to conventional wastewater treatment technologies. The production cost of MaB-flocs in aquaculture and food industry effluent is EUR 5.29 and 8.07kg⁻¹TSS, respectively. Capital costs and pond mixing costs are the major expenses. Commercializing MaB-flocs as aquaculture feed generates substantial revenues, but the largest profit potential lies in production of high-purity phycobiliproteins from MaB-flocs. These results highlight the large economic potential of MaB-floc technology, and justify its further development.

Environmental sustainability assessment of a microalgae raceway pond treating aquaculture wastewater: From up-scaling to system integration.

Bioresour Technol. 2015 Aug;190:321-31



Authors: Sfez S, Van Den Hende S, Taelman SE, De Meester S, Dewulf J

Abstract

The environmental sustainability of aquaculture wastewater treatment by microalgal bacterial flocs (MaB-flocs) in an outdoor raceway pond was analyzed using life cycle assessment. Pikeperch aquaculture wastewater treated at pilot scale (Belgium; 28m²) and industrial scale (hypothetical up-scaling; 41 ponds of 245m²) were compared. The integration of the MaB-floc raceway pond in a broader aquaculture waste treatment system was studied, comparing the valorisation of MaB-flocs as shrimp feed and as biogas. Up-scaling improves the resource footprint of the plant (848MJex,CEENEkg(-1) MaB-floc TSS at pilot scale and 277MJex,CEENEkg(-1) MaB-floc TSS at industrial scale) as well as its carbon footprint and eutrophication potential. At industrial scale, the valorisation of MaB-flocs as shrimp feed is overall more sustainable than as biogas but improvements should be made to reduce the energy use of the MaB-floc raceway pond, especially by improving the energy-efficiency of the pond stirring system.

Biology and Industrial Applications of Chlorella: Advances and Prospects.

Adv Biochem Eng Biotechnol. 2016;153:1-35

Authors: Liu J, Chen F

Abstract

Chlorella represents a group of eukaryotic green microalgae that has been receiving increasing scientific and commercial interest. It possesses high photosynthetic ability and is capable of growing robustly under mixotrophic and heterotrophic conditions as well. Chlorella has long been considered as a source of protein and is now industrially produced for human food and animal feed. Chlorella is also rich in oil, an ideal feedstock for biofuels. The exploration of biofuel production by Chlorella is underway. Chlorella has the ability to fix carbon dioxide efficiently and to remove nutrients of nitrogen and phosphorous, making it a good candidate for greenhouse gas biomitigation and wastewater bioremediation. In addition, Chlorella shows potential as an alternative expression host for recombinant protein production, though challenges remain to be addressed. Currently, omics analyses of certain Chlorella strains are being performed, which will help to unravel the biological implications of Chlorella and facilitate the future exploration of industrial applications.



Acute toxicity testing with the tropical marine copepod *Acartia sinjiensis*: optimisation and application.

Ecotoxicol Environ Saf. 2013 Nov;97:86-93

Authors: Gissi F, Binet MT, Adams MS

Abstract

Globally there is limited toxicity data for tropical marine species, and there has been a call for further research and development in the area of tropical marine ecotoxicology. An increase in developmental pressures in northern tropical Australia is causing a higher demand for toxicity test protocols with ecologically relevant species. Copepods are a diverse group of zooplankton that are major components of marine food webs. The calanoid copepod *Acartia sinjiensis* is widely distributed across tropical and sub-tropical brackish to marine waters of Australia and was identified in a recent comprehensive review of marine tropical toxicity testing in Australia as a suitable test organism. Through a number of optimisation steps including feeding trials, changes to culture and test conditions; a 48-h acute toxicity test with *A. sinjiensis* was modified to become a highly reliable and reproducible standard test protocol. Control mortality was improved significantly, and the sensitivity of *A. sinjiensis* to copper (EC₅₀ of 33µg/L), ammonia (EC₅₀ of 10mg/L) and phenol (EC₅₀ of 13mg/L) fell within the ranges of those reported previously, indicating that the modifications did not alter its sensitivity. In a comprehensive literature search we found that this species was the most sensitive to copper out of a range of marine copepods. The test was also successfully applied in toxicity assessments of four environmental samples: two produced formations waters (PFWs) and two mine tailing liquors (MTLs). The toxicity assessments utilised toxicity data from a suite of marine organisms (bacteria, microalgae, copepods, sea urchins, oysters, prawns, and fish). For the PFWs, which were predominantly contaminated with organic chemicals, *A. sinjiensis* was the most sensitive species (EC₅₀ value 2-17 times lower than for any other test species). For the predominantly metal-contaminated mine tailing liquors, its sensitivity was similar to that of other test species used. The modified 48-h acute toxicity test with *A. sinjiensis* proved to be a valuable tool in these toxicity assessments, and is recommended for use in tropical marine toxicity assessments for northern Australia.



Microalgal biomass production by using ultra- and nanofiltration membrane fractions of olive mill wastewater.

Water Res. 2013 Sep 01;47(13):4710-8

Authors: Cicci A, Stoller M, Bravi M

Abstract

Olive milling produces huge amounts of wastewater (OMWW) characterized by an extremely high organic load. Its polyphenols content is a hindrance to conventional biological treatment and to using it as growing medium for common microbial biomasses. The practice to dump it on soil is in conflict with the latest EU directives about waste management. OMWW can be effectively and efficiently treated by means of membrane technology to a fraction of the initial volume, but membrane processing concentrates still require treatment. Reversing the overall cost balance of membrane processing and subsequent treatment requires valorizing the concentrates through their reuse, as well as ensuring long-term service of the membrane system through effective wastewater pretreatment and sustainable, fouling-controlling, membrane operation conduite. Aim of this work is to reuse and valorize the ultra- and nanofiltration membrane concentrates as media for biomass production of microalgae and cyanobacteria. *Scenedesmus dimorphus* and *Arthrospira platensis*, usable as a food, feed, nutraceutical component or feedstock for biofuels, were selected for this investigation. Microalgal growth was experimentally determined and related to the composition of the concentrate-based media and to the irradiance distribution within the photobioreactor volume to decouple light limitation and medium chemical composition effects.

Characterization of sorption sites and differential stress response of microalgae isolates against tannery effluents from ranipet industrial area-An application towards phycoremediation.

Int J Phytoremediation. 2016 Aug 02;18(8):747-53

Authors: Balaji S, Kalaivani T, Sushma B, Pillai CV, Shalini M, Rajasekaran C

Abstract

Phycoremediation ability of microalgae namely *Oscillatoria acuminata* and *Phormidium irrigum* were validated against the heavy metals from tannery effluent of Ranipet industrial area. The microalgae species were cultured in media containing



tannery effluent in two different volumes and the parameters like specific growth rate, protein content and antioxidant enzyme activities were estimated. FTIR spectroscopy was carried out to know the sorption sites interaction. The antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) contents were increased in microalgae species indicating the free radical scavenging mechanism under heavy metal stress. SOD activity was 0.502 and 0.378 units/gram fresh weight, CAT activity was 1.36 and 0.256 units/gram fresh weight, GSH activity was 1.286 and 1.232 units/gram fresh weight respectively in the effluent treated microalgae species. Bio sorption efficiency for *Oscillatoria acuminata* and *Phormidium irrigum* was 90% and 80% respectively. FTIR analysis revealed the interaction of microalgae species with chemical groups present in the tannery effluent. From the results, the microalgae *Oscillatoria acuminata* possess high antioxidant activity and bio sorption efficiency when compared to *Phormidium irrigum* and hence considered useful in treating heavy metals contaminated effluents.

Phycoremediation of Tannery Wastewater Using Microalgae *Scenedesmus* Species.

Int J Phytoremediation. 2015;17(10):907-16

Authors: Ajayan KV, Selvaraju M, Unnikannan P, Sruthi P

Abstract

A number of microalgae species are efficient in removing toxicants from wastewater. Many of these potential species are a promising, eco-friendly, and sustainable option for tertiary wastewater treatment with a possible advantage of improving the economics of microalgae cultivation for biofuel production. The present study deals with the phycoremediation of tannery wastewater (TWW) using *Scenedesmus* sp. isolated from a local habitat. The test species was grown in TWW under laboratory conditions and harvested on the 12th day. The results revealed that the algal biomass during the growth period not only reduced the pollution load of heavy metals (Cr-81.2-96%, Cu-73.2-98%, Pb-75-98% and Zn-65-98%) but also the nutrients (NO₃ >44.3% and PO₄ >95%). Fourier Transform Infrared (FTIR) spectrums of *Scenedesmus* sp. biomass revealed the involvement of hydroxyl amino, carboxylic and carbonyl groups. The scanning electron micrograph (SEM) and Energy Dispersive X-ray Spectroscopic analysis (EDS) revealed the surface texture, morphology and element distribution of the biosorbent. Furthermore, the wastewater generated during wet-blue tanning process can support dense population of *Scenedesmus* sp.,



making it a potential growth medium for biomass production of the test alga for phycoremediation of toxicants in tannery wastewaters.

Characteristics and performance of aerobic algae-bacteria granular consortia in a photo-sequencing batch reactor.

J Hazard Mater. 2018 May 05;349:135-142

Authors: Liu L, Zeng Z, Bee M, Gibson V, Wei L, Huang X, Liu C

Abstract

The characteristics and performance of algae-bacteria granular consortia which cultivated with aerobic granules and targeted algae (*Chlorella* and *Scenedesmus*), and the essential difference between granular consortia and aerobic granules were investigated in this experiment. The result indicated that algae-bacteria granular consortia could be successfully developed, and the algae present in the granular consortia were mainly *Chlorella* and *Scenedesmus*. Although the change of chlorophyll composition revealed the occurrence of light limitation for algal growth, the granular consortia could maintain stable granular structure, and even showed better settling property than aerobic granules. Total nitrogen and phosphate in the algal-bacterial granular system showed better removal efficiencies (50.2% and 35.7%) than those in the aerobic granular system (32.8% and 25.6%) within one cycle (6 h). The biodiesel yield of aerobic granules could be significantly improved by algal coupled process, yet methyl linolenate and methyl palmitoleate were the dominant composition of biodiesel obtained from granular consortia and aerobic granules, respectively. Meanwhile, the difference of dominant bacterial communities in the both granules was found at the order level and family level, and alpha diversity indexes revealed the granular consortia had a higher microbial diversity.

Microbial community structures in high rate algae ponds for bioconversion of agricultural wastes from livestock industry for feed production.

Sci Total Environ. 2017 Feb 15;580:1185-1196

Authors: Mark Ibekwe A, Murinda SE, Murry MA, Schwartz G, Lundquist T



Abstract

Dynamics of seasonal microbial community compositions in algae cultivation ponds are complex. However, there is very limited knowledge on bacterial communities that may play significant roles with algae in the bioconversion of manure nutrients to animal feed. In this study, water samples were collected during winter, spring, summer, and fall from the dairy lagoon effluent (DLE), high rate algae ponds (HRAP) that were fed with diluted DLE, and municipal waste water treatment plant (WWTP) effluent which was included as a comparison system for the analysis of total bacteria, Cyanobacteria, and microalgae communities using MiSeq Illumina sequencing targeting the 16S V4 rDNA region. The main objective was to examine dynamics in microbial community composition in the HRAP used for the production of algal biomass. DNA was extracted from the different sample types using three commercially available DNA extraction kits; MoBio Power water extraction kit, Zymo fungi/bacterial extraction kit, and MP Biomedicals FastDNA SPIN Kit. Permutational analysis of variance (PERMANOVA) using distance matrices on each variable showed significant differences ($P=0.001$) in beta-diversity based on sample source. Environmental variables such as hydraulic retention time (HRT; $P<0.031$), total N ($P<0.002$), total inorganic N ($P<0.002$), total P ($P<0.002$), alkalinity ($P<0.002$), pH ($P<0.022$), total suspended solid (TSS; $P<0.003$), and volatile suspended solids (VSS; $P<0.002$) significantly affected microbial communities in DLE, HRAP, and WWTP. Of the operational taxonomic units (OTUs) identified to phyla level, the dominant classes of bacteria identified were: Cyanobacteria, Alpha-, Beta-, Gamma-, Epsilon-, and Delta-proteobacteria, Bacteroidetes, Firmicutes, and Planctomycetes. Our data suggest that microbial communities were significantly affected in HRAP by different environmental variables, and care must be taken in extraction procedures when evaluating specific groups of microbial communities for specific functions.

Enhanced biomass production through optimization of carbon source and utilization of wastewater as a nutrient source.

J Environ Manage. 2016 Dec 15;184(Pt 3):585-595

Authors: Gupta PL, Choi HJ, Pawar RR, Jung SP, Lee SM

Abstract

The study aimed to utilize the domestic wastewater as nutrient feedstock for mixotrophic cultivation of microalgae by evaluating appropriate carbon source. The microalgae *Chlorella vulgaris* was cultivated in municipal wastewater under various carbon sources (glucose, glycerol, and acetate), followed by optimization of



appropriate carbon source concentration to augment the biomass, lipid, and carbohydrate contents. Under optimized conditions, namely of 5 g/L glucose, *C. vulgaris* showed higher increments of biomass with 1.39 g/L dry cell weight achieving biomass productivity of 0.13 g/L/d. The biomass accumulated $19.29 \pm 1.83\%$ total lipid, $41.4 \pm 1.46\%$ carbohydrate, and $33.06 \pm 1.87\%$ proteins. Moreover, the cultivation of *Chlorella* sp. in glucose-supplemented wastewater removed 96.9% chemical oxygen demand, 65.3% total nitrogen, and 71.2% total phosphate. The fatty acid methyl ester obtained showed higher amount (61.94%) of saturated fatty acid methyl esters associated with the improved fuel properties. These results suggest that mixotrophic cultivation using glucose offers great potential in the production of renewable biomass, wastewater treatment, and consequent production of high-value microalgal oil.

Effect of aeration rate on performance and stability of algal-bacterial symbiosis system to treat domestic wastewater in sequencing batch reactors.

Bioresour Technol. 2016 Dec;222:156-164

Authors: Tang CC, Zuo W, Tian Y, Sun N, Wang ZW, Zhang J

Abstract

This study investigated aeration rate (0, 0.2, 0.4 and 1.0L/min) effects on algal-bacterial symbiosis (ABS) and conventional activated sludge (CAS) systems while treating domestic wastewater in sequencing batch reactors. Experiment results showed that ABS system performed better on NH_4^+-N , total nitrogen and total phosphorus removal than CAS system, especially under lower aeration rate condition (0.2Lair/min), with removal efficiencies improvements of 18.90%, 12.45% and 46.66%, respectively. The mechanism study demonstrated that a favorable aeration rate reduction (half of traditional value in CAS system) could enhance algae growth but weaken hydraulic shear force, which contributed to the interactions between algae and sludge flocs and further stability of ABS system. In addition, algae growth protected both ammonia and nitrite oxidizing bacteria from optical damage. It is expected that the present study would provide some new insights into ABS system and be helpful for development of low-energy demand wastewater treatment process.



Biochemical compositions and fatty acid profiles in four species of microalgae cultivated on household sewage and agro-industrial residues.

Bioresour Technol. 2016 Dec;221:438-446

Authors: Calixto CD, da Silva Santana JK, de Lira EB, Sassi PGP, Rosenhaim R, da Costa Sassi CF, da Conceição MM, Sassi R

Abstract

The potential of four regional microalgae species was evaluated in relation to their cell growth and biomass production when cultured in the following alternative media: bio-composts of fruit/horticultural wastes (HB), sugarcane waste and vinasse (VB) chicken excrements (BCE), raw chicken manure (RCM), and municipal domestic sewage (MDS). The cultures were maintained under controlled conditions and their growth responses, productivities, biochemical compositions, and the ester profiles of their biomasses were compared to the results obtained in the synthetic media. The MDS and HB media demonstrated promising results for cultivation, especially of *Chlorella* sp., *Chlamydomonas* sp., and *Lagerheimia longiseta*, which demonstrated productivities superior to those seen when grown on the control media. The highest lipid levels were obtained with the HB medium. The data obtained demonstrated the viability of cultivating microalgae and producing biomass in alternative media prepared from MDS and HB effluents to produce biodiesel.

Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource.

Bioresour Technol. 2015 May;184:179-189

Authors: Chiu SY, Kao CY, Chen TY, Chang YB, Kuo CM, Lin CS

Abstract

Using wastewater for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of nutrient addition, removing nitrogen and phosphorus from wastewater and producing microalgal biomass as bioresources for biofuel or high-value by-products. There are three main sources of wastewater, municipal (domestic), agricultural and industrial wastewater, which contain a variety of ingredients. Some components in the wastewater, such as nitrogen and phosphorus, are useful ingredients for microalgal cultures. In this review, the effects



on the biomass and lipid production of microalgal *Chlorella* cultures using different kinds of wastewater were summarized. The use of the nutrients resource in wastewater for microalgal cultures was also reviewed. The effect of ammonium in wastewater on microalgal *Chlorella* growth was intensively discussed. In the end, limitations of wastewater-based of microalgal culture were commented in this review article.

Lipid production by a mixed culture of oleaginous yeast and microalga from distillery and domestic mixed wastewater.

Bioresour Technol. 2014 Dec;173:132-139

Authors: Ling J, Nip S, Cheok WL, de Toledo RA, Shim H

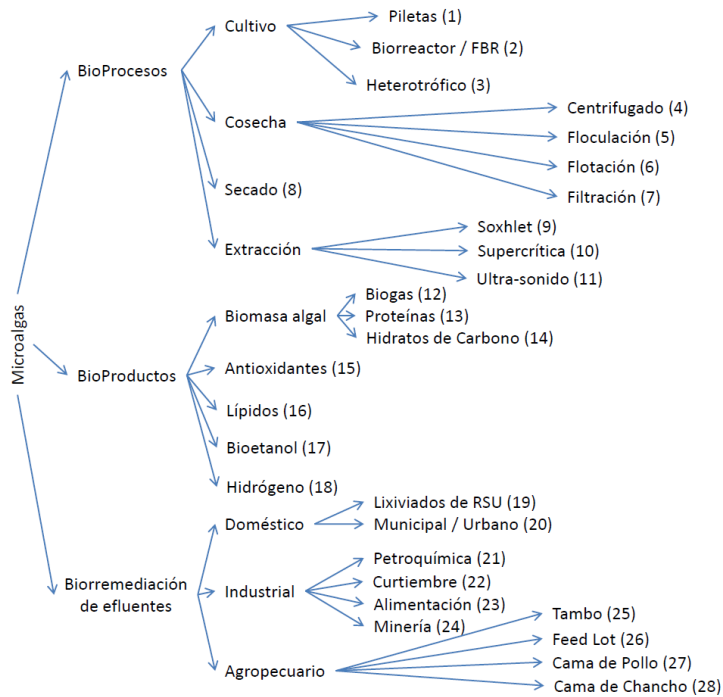
Abstract

Lipid productivity by mixed culture of *Rhodospiridium toruloides* and *Chlorella pyrenoidosa* was studied using 1:1 mixed real wastewater from distillery and local municipal wastewater treatment plant with initial soluble chemical oxygen demand (SCOD) around 25,000 mg/L, initial cell density of 2×10^7 cells/mL (yeast) and 5×10^6 cells/mL (microalga), at 30 °C and 2.93 W/m² (2000 lux, 12:12 h light and dark cycles). Lipid content and lipid yield achieved were $63.45 \pm 2.58\%$ and 4.60 ± 0.36 g/L with the associated removal efficiencies for SCOD, total nitrogen (TN), and total phosphorus (TP) at $95.34 \pm 0.07\%$, $51.18 \pm 2.17\%$, and $89.29 \pm 4.91\%$, respectively, after 5 days of cultivation without the pH adjustment. Inoculation of microalgae at 40 h of the initial yeast cultivation and harvesting part of inactive biomass at 72 h by sedimentation could improve both lipid production and wastewater treatment efficiency under non-sterile conditions.

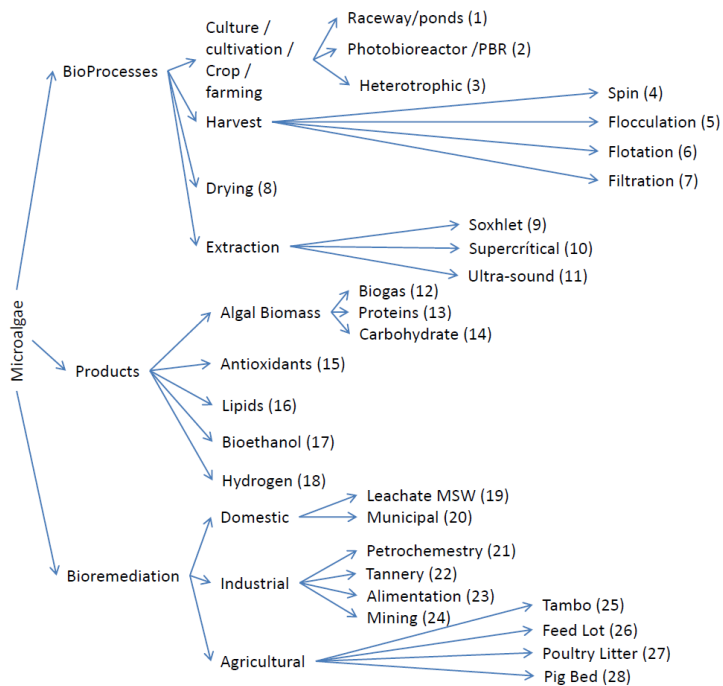


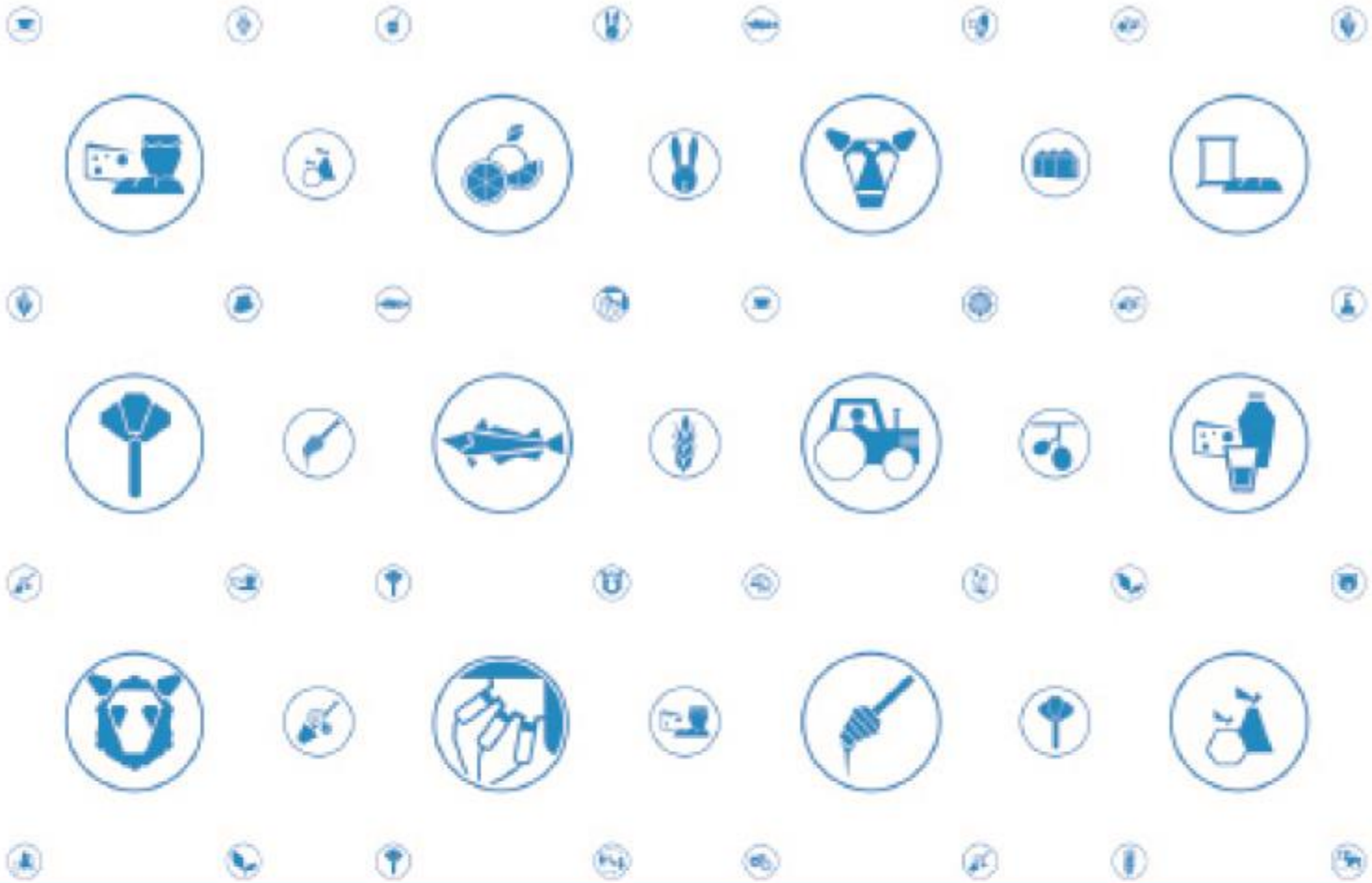
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