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Microalgas

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PUBLICACIONES

A biorefinery for valorization of industrial waste-water and flue gas by microalgae for waste mitigation, carbon-dioxide sequestration and algal biomass production.

Sci Total Environ. 2019 Oct 20;688:129-135

Authors: Yadav G, Dash SK, Sen R

Abstract

Massive industrialization all over the globe is the main cause for the generation of huge quantity of wastes such as flue gas and wastewaters. Mindless release of these hazardous wastes into the environment is threatening the health and survival of the mankind. Judicious use of these wastes for microalgal biomass cultivation is recognized as a plausible approach for the creation of a renewable and sustainable process chain for biofuel production. This study was designed to cultivate microalgae utilizing the organic and inorganic nutrients from the industrial wastewater (IWW) and coal-fired flue gas (FG) for simultaneous waste bio-remediation and biomass production for biorefinery application in closed photobioreactors. The two microalgae, *Chlorella* sp. and *Chlorococcum* sp. were cultivated in industrial wastewater where varying concentrations of coal-fired FG from 1 to 10% CO₂, volume/volume percent (v/v) was supplied to stimulate the mixotrophic growth. Performance of the two microalgae was evaluated in terms of nutrient removal (ammonium, nitrate, phosphate and COD), CO₂ fixation, total lipid and carbohydrate content obtained in the integrated mode of process development. The IWW with flue gas (5% CO₂ (v/v)) resulted in maximum growth and CO₂ fixation. The highest biomass growth (1.52 g L⁻¹) and CO₂ fixation (187.65 mg L⁻¹ d⁻¹) of *Chlorella* sp. with nutrient removal of >70% was observed by 5th day of batch cultivation. Nearly 90% removal of nitrogen resulted in nutrient limitation condition that steered the accumulation of lipid (17-34%) and carbohydrate (21.5-23.1%) in *Chlorella* and *Chlorococcum* sp. An overall 1.7 fold improvement in biomass was observed in this process integration compared with control culture. The present study presents a green process for waste remediation, CO₂ fixation and production of biomass rich in lipid & carbohydrate content for the development of a green microalgal biorefinery.

Performance evaluation of a control strategy for photosynthetic biogas upgrading in a semi-industrial scale photobioreactor.

Bioresour Technol. 2020 Jul;307:123207

Authors: Rodero MDR, Carvajal A, Arbib Z, Lara E, de Prada C, Lebrero R, Muñoz R

Abstract

The validation of a control strategy for biogas upgrading via light-driven CO₂ consumption by



microalgae and H₂S oxidation by oxidizing bacteria using the oxygen photosynthetically generated was performed in a semi-industrial scale (9.6 m³) photobioreactor. The control system was able to support CO₂ concentrations lower than 2% with O₂ contents ≤ 1% regardless of the pH in the cultivation broth (ranging from 9.05 to 9.50). Moreover, the control system was efficient to cope with variations in biogas flowrate from 143 to 420 L h⁻¹, resulting in a biomethane composition of CO₂ < 2.4%, CH₄ > 95.5%, O₂ < 1% and no H₂S. Despite the poor robustness of this technology against failures in biogas and liquid supply (CH₄ concentration of 67.5 and 70.9% after 2 h of biogas or liquid stoppage, respectively), the control system was capable of restoring biomethane quality in less than 2 h when biogas or liquid supply was resumed.

Microalgae Cultivation and Biomass Quantification in a Bench-Scale Photobioreactor with Corrosive Flue Gases.

J Vis Exp. 2019 Dec 19;(154):

Authors: Molitor HR, Williard DE, Schnoor JL

Abstract

Photobioreactors are illuminated cultivation systems for experiments on phototrophic microorganisms. These systems provide a sterile environment for microalgal cultivation with temperature, pH, and gas composition and flow rate control. At bench-scale, photobioreactors are advantageous to researchers studying microalgal properties, productivity, and growth optimization. At industrial scales, photobioreactors can maintain product purity and improve production efficiency. The video describes the preparation and use of a bench-scale photobioreactor for microalgal cultivation, including the safe use of corrosive gas inputs, and details relevant biomass measurements and biomass productivity calculations. Specifically, the video illustrates microalgal culture storage and preparation for inoculation, photobioreactor assembly and sterilization, biomass concentration measurements, and a logistic model for microalgal biomass productivity with rate calculations including maximum and overall biomass productivities. Additionally, since there is growing interest in experiments to cultivate microalgae using simulated or real waste gas emissions, the video will cover the photobioreactor equipment adaptations necessary to work with corrosive gases and discuss safe sampling in such scenarios.

The effect of the microalgae-bacteria microbiome on wastewater treatment and biomass production.

Appl Microbiol Biotechnol. 2020 Jan;104(2):893-905

Authors: Paddock MB, Fernández-Bayo JD, VanderGheynst JS



Abstract

The use of microalgae for wastewater treatment has been proposed as a cost-effective method to produce biofuels while remediating waste streams. This study examined the microalgae biomass production rate, wastewater treatment efficiency, and prokaryotic organism microbiome associated with microalgae *Chlorella sorokiniana* cultivated on anaerobic digester effluent. Final microalgae biomass concentrations from nine photobioreactors were highly variable and had values that ranged between 0.14 g/L and 0.90 g/L. Nutrient removal efficiencies for TN (total nitrogen), N-NH₄ (ammonium nitrogen), and COD (chemical oxygen demand) ranged from 34% to 67%, 65% to 97%, and 60% to 14%, respectively. Analysis of individual OTUs (operational taxonomic units) from the microbial community revealed that microalgae biomass concentrations were significantly correlated with the relative abundance of OTUs in the genus *Pseudomonas*. Predictive metagenomic analyses identified additional correlations associated with biomass production and nutrient removal. These results suggest that the microbial community present during microalgae cultivation on wastewater can impact the performance of the system for biomass production and wastewater treatment.

A simplistic approach of algal biofuels production from wastewater using a Hybrid Anaerobic Baffled Reactor and Photobioreactor (HABR-PBR) System.

PLoS One. 2019;14(12):e0225458

Authors: Khalekuzzaman M, Alamgir M, Islam MB, Hasan M

Abstract

The current technologies of algal biofuels production and wastewater treatment (e.g., aerobic) process are still in question, due to the significant amount of fresh water and nutrients requirements for microalgae cultivation, and negative energy balance in both processes, especially when considered in the context of developing countries around the world. In this research, a simplistic sustainable approach of algal biofuels production from wastewater was proposed using a Hybrid Anaerobic Baffled Reactor (HABR) and Photobioreactor (PBR) system. The study suggests that the HABR was capable of removing most of the organic and solid (>90% COD and TSS removal) from wastewater, and produced a healthy feedstock (high N: P = 3:1) for microalgae cultivation in PBRs for biofuels production. A co-culture of *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus dimorphus* showed high lipid content up to 44.1%; and the dominant FAMES composition (C16-C18) of 87.9% in produced biofuels. Perhaps, this proposed low-cost technological approach (e.g., HABR-PBR system) would connect the currently broken link of sustainable bioenergy generation and wastewater treatment pathway for developing countries.



Sustainable production of bio-crude oil via hydrothermal liquefaction of symbiotically grown biomass of microalgae-bacteria coupled with effective wastewater treatment.

Sci Rep. 2019 Oct 18;9(1):15016

Authors: Goswami G, Makut BB, Das D

Abstract

The study demonstrates a sustainable process for production of bio-crude oil via hydrothermal liquefaction of microbial biomass generated through co-cultivation of microalgae and bacteria coupled with wastewater remediation. Biomass concentration and wastewater treatment efficiency of a tertiary consortium (two microalgae and two bacteria) was evaluated on four different wastewater samples. Total biomass concentration, total nitrogen and COD removal efficiency was found to be 3.17 g L⁻¹, 99.95% and 95.16% respectively when consortium was grown using paper industry wastewater in a photobioreactor under batch mode. Biomass concentration was enhanced to 4.1 g L⁻¹ through intermittent feeding of nitrogen source and phosphate. GC-MS and FTIR analysis of bio-crude oil indicates abundance of the hydrocarbon fraction and in turn, better oil quality. Maximum distillate fraction of 30.62% lies within the boiling point range of 200-300 °C depicting suitability of the bio-crude oil for conversion into diesel oil, jet fuel and fuel for stoves.

Kinetic study of nutrients removal from municipal wastewater by *Chlorella vulgaris* in photobioreactor supplied with CO₂-enriched air.

Environ Technol. 2020 Feb;41(5):617-626

Authors: Chaudhary R, Tong YW, Dikshit AK

Abstract

The microalgae *Chlorella vulgaris* ATCC 13482 was used in the present study for municipal wastewater treatment. Batch experiments were performed in bubble column photobioreactors of 7 L working volume maintained at 25 ± 2°C and 14 h/10 h of photo and dark cycle. The treatment process was enhanced by using CO₂-augmented air (5% CO₂ v/v) supply into the microalgal culture in comparison to the use of normal air (0.03% CO₂ v/v). For a period of 7 days, *C. vulgaris* effected maximum removals of 74.4% soluble fraction of chemical oxygen demand, 72% ammonia (NH₄-N), 60% nitrate (NO₃-N) and 81.93% orthophosphate (PO₄-P) with use of normal air, whereas 84.6% sCOD, 88% NH₄-N, 72% NO₃-N and 92.8% PO₄-P removals, respectively, with use of 5% CO₂/air supply. Using kinetic study data, the specific rates of ammonia and phosphate uptake (q_{ammonia} and q_{phosphate}) by *C. vulgaris* at 5% CO₂/air supply were found to be 2.41 and 0.85 d⁻¹, respectively. Using the algal remediation technology, nitrogen-phosphorus-potassium recovery from sewage treatment plant of 37.5 million litres per day wastewater influent capacity was calculated to be ~298.5, 55.4 and 83.7 kg d⁻¹, respectively.



Biological Carbon Recovery from Sugar Refinery Washing Water into Microalgal DHA: Medium Optimization and Stress Induction.

Sci Rep. 2019 Dec 27;9(1):19959

Authors: Moon M, Park WK, Suh WI, Chang YK, Lee B

Abstract

Sugar refinery washing water (SRWW) contains abundant levels of carbon sources and lower levels of contaminants than other types of wastewater, which makes it ideal for heterotrophic cultivation of microalgae. Here, carbon sources in SRWW were utilized for conversion into the form of value-added docosahexaenoic acid (DHA) using *Aurantiochytrium* sp. KRS101. Since SRWW is not a defined medium, serial optimizations were performed to maximize the biomass, lipid, and DHA yields by adjusting the nutrient (carbon, nitrogen, and phosphorus) concentrations as well as the application of salt stress. Optimum growth performance was achieved with 30% dilution of SRWW containing a total organic carbon of 95,488 mg L⁻¹. Increasing the nutrient level in the medium by supplementation of 9 g L⁻¹ KH₂PO₄ and 20 g L⁻¹ yeast extract further improved the biomass yield by an additional 14%, albeit at the expense of a decrease in the lipid content. Maximum biomass, lipid, and DHA yields (22.9, 6.33, and 2.03 g L⁻¹, respectively) were achieved when 35 g L⁻¹ sea salt was applied on a stationary phase for osmotic stress. These results demonstrate the potential of carbon-rich sugar refinery washing water for DHA production using *Aurantiochytrium* sp. KRS101 and proper cultivation strategy.

High fatty acid productivity from *Scenedesmus obliquus* in heterotrophic cultivation with glucose and soybean processing wastewater via nitrogen and phosphorus regulation.

Sci Total Environ. 2020 Mar 15;708:134596

Authors: Shen XF, Gao LJ, Zhou SB, Huang JL, Wu CZ, Qin QW, Zeng RJ

Abstract

In this study, the effects of nitrogen and phosphorus supply on biodiesel production from *Scenedesmus obliquus* with glucose as the carbon source were investigated. It was found that sufficient phosphorus could further improve biodiesel production under nitrogen starvation. *S. obliquus* was cultivated in soybean processing wastewater. The removal efficiencies of carbon oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) after 8-day cultivation were 72%, 95%, and 54%, respectively. Moreover, the fatty acid productivity after eight-day cultivation reached as high as 99.3 mg·L⁻¹·d⁻¹, which was 1.15 times higher than the highest efficiency using a glucose culture. This result was due to two naturally-formed stages occurring with sufficient phosphorus: nitrogen sufficiency stage for biomass and nitrogen starvation stage for lipid accumulation. It verified the conclusion of the roles of nitrogen and phosphorus obtained in the glucose culture and provided an economic and environmentally friendly choice for biodiesel production with efficient soybean wastewater treatment.



Heterotrophic cultivation of *Chlorella vulgaris* using saline waste water from the demineralization of cheese whey.

Biotechnol Lett. 2020 Feb;42(2):209-217

Authors: Ghobrini D, Potocar T, Smolova J, Krausova G, Yakoub-Bougdal S, Branyik T

Abstract

OBJECTIVE: Desalination of cheese whey by electrodialysis yields saline wastewater (SWW). The goal was to test this as the basis of a culture medium and to prove experimentally the concept that it was a suitable resource for heterotrophic cultivation of the freshwater green microalga *Chlorella vulgaris*.

RESULTS: Optimization of glucose concentration, nitrogen source and medium salinity for microalgal growth was first carried out in defined medium (DM) and shake flasks. These results were then adopted in shake flask cultivation experiments using pre-treated SWW medium (PSWW). Subsequently, microalgal growth under optimized conditions was tested in bioreactors. Various media such as DM, PSWW and diluted PSWW (DPSWW) were compared. Volumetric biomass productivities decreased in the order DM (0.371 g L⁻¹ h⁻¹, urea) > DPSWW (0.315 g L⁻¹ h⁻¹, soy peptone) > PSWW (0.152 g L⁻¹ h⁻¹, soy peptone). Although biomass productivities in DPSWW and PSWW media were significantly lower than in DM, these media required the addition of only 66 and 33% of DM N sources, respectively. No other added DM component was necessary in (D)PSWW to achieve microalgal growth.

CONCLUSIONS: Although the optimized cultivation of freshwater microalgae on alternative medium based on SWW resulted in biomass productivities lower than those on DM, the required addition of N sources was also lower. Potentially lower production costs of *Chlorella* biomass and the meaningful use of SWW are the main outcomes of this work.

Subtopic: Advances in water and wastewater treatment harvesting of *Chlorella* sp. microalgae using *Aspergillus niger* as bio-flocculant for aquaculture wastewater treatment.

J Environ Manage. 2019 Nov 01;249:109373

Authors: Mohd Nasir N, Mohd Yunus FH, Wan Jusoh HH, Mohammad A, Lam SS, Jusoh A

Abstract

Microalgae have been increasingly used to generate biofuel, thus a sustainable technique should be implemented to harvest the biomass to ensure its existence in the environment. *Aspergillus niger* was used as bio-flocculant to harvest microalgae from aquaculture wastewater via flocculation technique over a range of pH and mixing rate. The bio-flocculant showed ability to adapt at a wide range of pH from 3.0 to 9.0 and at a mixing rate of 100-150 rpm, producing a harvesting efficiency of higher than 90%. The treated water possessed low concentration of chlorophyll-a (0.3-0.6 mg L⁻¹) and cell density (2 × 10⁶-3 × 10⁶ cell mL⁻¹). These indicate that



Aspergillus niger is a promising bio-flocculant to be used in harvesting microalgae, thus promoting the use of flocculation as a green technology in aquaculture wastewater treatment.

Auto-flocculation microalgae species *Tribonema* sp. and *Synechocystis* sp. with T-IPL pretreatment to improve swine wastewater nutrient removal.

Sci Total Environ. 2020 Apr 07;725:138263

Authors: Cheng P, Chen D, Liu W, Cobb K, Zhou N, Liu Y, Liu H, Wang Q, Chen P, Zhou C, Ruan R

Abstract

It is recognized coupling microalgae, which is rich in lipids or protein with wastewater treatment offers extra economic benefits that can potentially make microalgal production feasible by reducing production costs and providing environmental benefits. However, the pretreatment of high concentration nutrients such as ammonia nitrogen (NH₃-N), total phosphorus (TP) and chemical oxygen demand (COD) in swine wastewater is the premise of application for microalgae in wastewater treatment. This study two auto-flocculation microalgae *Tribonema* sp. and *Synechocystis* sp. were selected for evaluation; they were cultivated in diluted swine wastewater together after it was pretreated with titanium dioxide (TiO₂) plus intense pulsed light (T-IPL). The results showed that the growth of the two strains in the wastewater pretreated with T-IPL grew better than when grown without the pretreatment. The content of lipid in the two algae, cultured in the pretreated wastewater, was also higher than the lipid content from the un-pretreated wastewater; but protein content was lower. Overall, the removal efficiencies of pollutants NH₃-N, TP, and COD by the two microalgae in anaerobic digestion of swine wastewater (ADSW) with T-IPL pretreatment, were higher than the removal efficiencies without pretreatment. This research also indicates that these two auto-flocculation microalgae have the potential to reduce harvesting costs. And, using T-IPL to pretreat wastewater could provide a promising method for the pretreatment of wastewater.

Characterization of Soluble Algal Products (SAPs) after electrocoagulation of a mixed algal culture.

Biotechnol Rep (Amst). 2020 Mar;25:e00433

Authors: Rafiee P, Ebrahimi S, Hosseini M, Tong YW

Abstract

The dewatering of algal culture requires coagulation of the algal cells. However, the coagulation in a continuous operation is slowed down through the excretion of Soluble Algal Products (SAPs). Electrocoagulation (EC), already utilized as a coagulation technique, has been investigated for its effects on SAPs characterizations. A mixed culture of *Chlorella vulgaris*,



Scenedesmus Obliquus, *Botryococcus braunii*, *Botryococcus sudeticus*, and *Afrocarpus falcatus* was prepared and SAPs characteristics, including Specific Ultra Violet Absorbance (SUVA), Zeta potential, Molecular Weight (MW) fractionation, Dissolved Organic Carbon (DOC), protein and carbohydrate content, Excitation-Emission Matrix, and hydrophobicity using XAD resins, were measured and evaluated before and after electrocoagulation using mild steel and aluminum electrodes at 5 and 10 min. The results showed several improvements after EC. According to results, EC can render SAPs hydrophobicity up to 95 %, and the fluorescence peak results showed the complete removal of humic-like. Moreover, the SAPs were removed up to 21, 60, and 47 % for protein, carbohydrate and DOC, respectively. Results collectively showed that electrocoagulation might be able to mitigate the negative effects of growth on flocculation.

Analysis of the energy barrier between *Chlorella vulgaris* cells and their interfacial interactions with cationic starch under different pH and ionic strength.

Bioresour Technol. 2020 May;304:123012

Authors: Wei C, Huang Y, Liao Q, Xia A, Zhu X, Zhu X

Abstract

To explore the energy barrier between microalgae cells that impedes their aggregation and their interfacial interactions with cationic starch (CS), this study applied the extended Derjaguin Landau Verwey Overbeek (eDLVO) theory combined with the flocculation performance to analyze the interactions. The result shows that zeta potential based electrostatic interaction played a determinative role no matter for the energy barrier or the interfacial interactions. The energy barrier between microalgae cells would decrease with the descend of the pH and it disappeared when the pH decreased to 3 and resulted in self-flocculation. The quantitative analysis of the interfacial interactions between microalgae cell and CS showed well agreement with the experiment data of flocculation efficiency (FE) under different conditions of pH and ionic strength. Thus, the quantitative findings will be helpful to know the aggregation and flocculation process better and find more effective flocculants for microalgae harvesting.

Nutrients removal from piggery wastewater coupled to lipid production by a newly isolated self-flocculating microalga *Desmodesmus sp.* PW1.

Bioresour Technol. 2020 Apr;302:122806

Authors: Chen Z, Shao S, He Y, Luo Q, Zheng M, Zheng M, Chen B, Wang M

Abstract

A newly isolated microalgal strain, *Desmodesmus sp.* PW1, possessing not only high potential for removing nitrogen and phosphorous from piggery wastewater but excellent self-flocculating



ability, was provided here. Strain PW1 grew well in diluted and undiluted piggery wastewater, and could effectively remove total nitrogen and total phosphorus with removal rates up to 90% and 70%, respectively. In the laboratory scale by 30-L photobioreactor, microalga also performed well in TN (65.3%) and TP (83.5%) removal. Strain PW1 cultivated in the stationary phase achieved high self-flocculating efficiency (>90%) in 2.5 h of settling; meanwhile, temperature and pH slightly influenced on the flocculation. The potential mechanism on self-flocculation was considered related to hydrophobic extracellular polymeric substances. Furthermore, the fatty acid compositions of PW1 were mainly hexadecanoic acid, oleic acid and linoleic acid. Taken together, *Desmodesmus* sp. PW1 was the promising candidate to overcome the microalgae harvesting problem in piggery wastewater treatment.

Harvesting of freshwater microalgae *Scenedesmus* sp. by electro-coagulation-flocculation for biofuel production: effects on spent medium recycling and lipid extraction.

Environ Sci Pollut Res Int. 2020 Jan;27(3):3497-3507

Authors: Pandey A, Shah R, Yadav P, Verma R, Srivastava S

Abstract

There is growing interest in recent times for microalgae as a sustainable energy source. However, efficient harvesting of microalgal biomass for various industrial applications is still considered a bottleneck. The present study attempts to evaluate microalgae *Scenedesmus* sp. harvesting using electro-coagulation-flocculation (ECF). Plackett-Burman design was exploited to explore the significant process parameters, whereas Taguchi's array design was employed for optimization. The optimal conditions were optimized as initial pH 5.0, electrolysis time 15 min, electrode distance 2 cm, sedimentation time 60 min, and current density 12 mA cm⁻² for complete harvesting. Under optimum conditions, the energy utilization and the operation cost of ECF process was estimated to be 2.65 kWh kg⁻¹ and USD 0.29 kg⁻¹, respectively. Thus, ECF-based microalgae harvesting was found as a low-cost technique. In addition, neutralizing pH and supplementing macro- and micronutrients enabled the flocculated medium to maintain an approximate growth yield in algal cultivation to that of the fresh BG11 medium. ECF did not affect the amount of microalgal lipids (28.6 ± 1.2, % wt.), chlorophyll a (8.3 ± 0.3 µg mL⁻¹), and fatty acid methyl ester composition (C15:0, C16:0, C17:0, and C18:0) as well. These results strongly recommend ECF as the most appropriate and promising method for harvesting *Scenedesmus* sp. for biofuel production.

Biomitigation of CO₂ from flue gas by *Scenedesmus obtusiusculus* AT-UAM using a hybrid photobioreactor coupled to a biomass recovery stage by electro-coagulation-flotation.

Environ Sci Pollut Res Int. 2020 Mar 04;:



Abstract

The microalga *Scenedesmus obtusiusculus* AT-UAM efficiently captured CO₂ from two flue gas streams in a hybrid photobioreactor located in a greenhouse. Uptake rates of CO₂, NO, and SO₂ from a formulated gas stream were 160.7 mg L⁻¹ day⁻¹, 0.73 mg L⁻¹ day⁻¹, and 1.56 mg L⁻¹ day⁻¹, respectively, with removal efficiencies of 100% for all gases. Exhaust gases of a motor generator were also removed with uptake rates of 111.4 mg L⁻¹ day⁻¹, 0.42 mg L⁻¹ day⁻¹, and 0.98 mg L⁻¹ day⁻¹, obtaining removal efficiencies of 77%, 71%, and 53% for CO₂, NO_x, and SO₂, respectively. On average, 61% of the CO₂ from both flue gas streams was assimilated as microalgal biomass. The maximum CO₂ uptake rate of 182 mg L⁻¹ day⁻¹ was achieved for formulated flue gas flow rate above 100 mL min⁻¹. The biomass recovery of 88% was achieved using a 20-L electro-coagulation-flotation chamber coupled to a settler with a low specific power consumption of 0.27 kWh kg⁻¹. The photobioreactor was operated for almost 7 months without contamination of invasive species or a decrease in the activity. It is a very encouraging result for long-term operation in flue gas treatment.

Microalgae harvesting from wastewater by pH modulation and flotation: Assessing and optimizing operational parameters.

J Environ Manage. 2020 Jan 15;254:109825

Authors: Leite LS, Dos Santos PR, Daniel LA

Abstract

Microalgae harvesting is one of the major bottlenecks for the production of high-value microalgal products on a large scale, which encourages investigations of harvesting methods with better cost-benefits. Among these harvesting techniques, flotation stands out as a promising method, however it is still minimally explored when compared to the sedimentation method. In this study, the pH modulation followed by dissolved air flotation (DAF) was tested as a harvesting method for *Chlorella sorokiniana* cultivated in wastewater. The main aims of this study were to optimize the operational parameters of coagulation (pH, velocity gradient, and mixing time) and flotation (recirculation rate), check their reproducibility and resilience with the variability of wastewater characteristics, and evaluate the final wastewater quality after treatment using an optimized harvesting method. Parameter optimization was carried out using the one-factor-at-a-time method. The optimal parameters were a velocity gradient of 500 s⁻¹, mixing time of 30 s, pH 12, and 20% of recirculation rate. High efficiencies were obtained for *C. sorokiniana* removal (96.5-97.9%), making it a successful process. Moreover, the photobioreactor effluent quality was also improved significantly after microalgae harvesting, with high nutrient removal (88.6-95.1% of total Kjeldahl nitrogen and 91.8-98.3% of total phosphorus) and organic matter removal (80.5-86.8% of chemical oxygen demand). The results showed the pH modulation and DAF as an effective process for wastewater treatment and biomass harvesting. This study also indicated the importance of operational optimization, not studied until now, in which the achieved results could be potentially applied as practical guidelines for microalgae harvesting on a large scale.



Microalgae harvesting with the novel flocculant hairy cationic nanocrystalline cellulose.

Colloids Surf B Biointerfaces. 2019 Jun 01;178:329-336

Authors: Lopez-Exposito P, Campano C, van de Ven TGM, Negro C, Blanco A

Abstract

This paper investigates the flocculation of *Chlorella sorokiniana* suspensions with a novel cellulose derivative, namely hairy cationic nanocrystalline cellulose (CNCC). CNCC are a brand new family of nanocellulose characterized by having two positively charged amorphous ends joint through a common crystalline shaft. Flocculation was monitored through laser reflectance and its mechanism was studied by means of zeta potential, fractal dimension and turbidity removal. CNCC dosage and shear rate were varied and their effect on floc morphology and filterability were assessed. CNCC effectively flocculated the cultures at dosages well below and over the isoelectric point, being the flocculation mechanisms and floc strength highly dependent on the doses applied. The filtration propensity of flocculated suspensions proved highly sensitive to small differences in flocs' geometry. The aggregation process entailed two phases, a first one in which the CNCC adsorbed on the surface of microalgal cells according to a flat random deposition up to reaching a maximal cell coverage, and a second one in which the free spots left were progressively covered with orthogonally deposited CNCC, being this later configuration the main responsible for intercellular attachment. The present work demonstrates that CNCC is an effective flocculant of microalgal cell suspensions and constitutes an alternative worth exploring for the aggregation of other cells' suspensions.

A highly efficient and energy-saving magnetically induced membrane vibration system for harvesting microalgae.

Bioresour Technol. 2020 Mar;300:122688

Authors: Zhao Z, Mertens M, Li Y, Muylaert K, Vankelecom IFJ

Abstract

The optimal operational parameters of a second generation magnetically induced membrane vibration (MMV) system were determined using the response surface methodology (RSM) combined with single-factor experiments. The membrane surfaces were characterized by scanning electron microscopy (SEM) and algae cell states by inverted microscopy. The effect of an intermittent vibration strategy on filtration performance and energy consumption was studied. The results showed that the responses could be fitted by RSM models. High membrane flux, low energy consumption, efficient fouling control and no damage to the microalgae could thus be realized. The filtration strategy tests suggested that an intermittent cycle time of 4 min with 50% vibration rate could be the best vibration strategy for harvesting the microalgae under investigation.



Effects of sugarcane bagasse hydrolysate (SCBH) on cell growth and fatty acid accumulation of heterotrophic *Chlorella protothecoides*.

Bioprocess Biosyst Eng. 2019 Jul;42(7):1129-1142

Authors: Chen JH, Liu L, Lim PE, Wei D

Abstract

Microalgal lipid production by *Chlorella protothecoides* using sugarcane bagasse hydrolysate was investigated in this study. First, maximum glucose and reducing sugar concentrations of 15.2 and 27.0 g/L were obtained in sugarcane bagasse hydrolysate (SCBH), and the effects of different percentages of glucose and xylose on algal cultivation were investigated. Afterwards, SCBH was used as a carbon source for the cultivation of *C. protothecoides* and higher biomass concentration of 10.7 g/L was achieved. Additionally, a large amount of fatty acids, accounting up to 16.8% of dry weight, were accumulated in *C. protothecoides* in the nitrogen-limited (0.1-1 mmol/L) culture. Although SCBH inhibited fatty acid accumulation to a certain degree and the inhibition was aggravated by nitrogen starvation, SCBH favored microalgal cell growth and fatty acid production. The present study is of significance for the integration of cost-effective feedstocks production for biodiesel with low-cost SCBH as well as environmentally friendly disposal of lignocellulosic wastes.

Microalgae based biofertilizer: A life cycle approach.

Sci Total Environ. 2020 Mar 23;724:138138

Authors: Castro JS, Calijuri ML, Ferreira J, Assemany PP, Ribeiro VJ

Abstract

Waste, especially biomass in general, is a large reservoir of nutrients that can be recovered through different technologies and used to produce biofertilizers. In the present study, environmental impacts of the production of microalgae biomass-based phosphate biofertilizer compared to triple superphosphate through life-cycle assessment conducted in the Simapro® software were investigated. The functional unit of the analysis was 163 g of P for both fertilizers. Phosphorus was recovered from a meat processing industry effluent in a high-rate algal pond. Impacts related to the entire biofertilizer chain impacted mainly on climate changes (3.17 kg CO₂eq). Microalgae biofertilizer had higher environmental impact than conventional fertilizer in all impact categories, highlighting climate change and terrestrial ecotoxicity. An ideal scenario was created considering that: all energy used comes from photovoltaic panels; in the separation step a physical method will be used, without energy expenditure (i.e. gravimetric sedimentation) and; biomass will be dried in a drying bed instead of the thermal drying. In this scenario, the impact of biofertilizer approached considerably those of triple superphosphate. When impacts of biomass cultivation and concentration stages were disregarded, drying step was of great relevance, contributing to increase biofertilizer impacts. More research is needed to



optimize the algae production chain and determine the possibility of obtaining higher added value products more environmental attractive.

Enhanced yield of *Scenedesmus obliquus* biomacromolecules through medium optimization and development of microalgae based functional chocolate.

J Food Sci Technol. 2020 Mar;57(3):1090-1099

Authors: Hlaing SAA, Sadiq MB, Anal AK

Abstract

The freshwater green microalga *Scenedesmus obliquus* was cultivated to enhance the contents of proteins, carbohydrates and lipids by using Box-Behnken experimental design. *S. obliquus* was cultured under phototrophic conditions by using Bold's Basal Medium with different cultivation parameters including pH (7, 8 and 9), salinity (10, 30 and 50 mM), and nitrogen source (0.125, 0.5 and 1 g/L). The highest biomass yield (64.9 ± 0.94 mg/L/day) was obtained by using optimized medium at a salinity concentration of 30 mM (w/v), and nitrogen sources of 0.125 g/L. The maximum content of protein, lipid and carbohydrates from *S. obliquus* optimized medium were 342.19 ± 0.28 mg/g, 241.41 ± 4.32 mg/g and 288.05 ± 1.12 mg/g of dry wt. respectively. The amino acid and fatty acid analysis of *S. obliquus* biomass indicated the presence of significant amount of essential amino acids and essential fatty acids. Furthermore, chocolate crispy bar was developed by fortification with encapsulated freeze-dried *S. obliquus* and evaluated for its oxidative stability and sensory analysis. The chocolate fortified with microalgae can be a potential source of essential fatty acids and amino acids in addition to other bioactive compounds.

Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L.

Sci Rep. 2020 Feb 18;10(1):2820

Authors: Mutale-Joan C, Redouane B, Najib E, Yassine K, Lyamlouli K, Laila S, Zeroual Y, Hicham EA

Abstract

The present study investigates the biostimulant effects of 18 Crude Bio-Extracts (CBEs) obtained from Microalgae and Cyanobacteria on tomato plant growth, chlorophyll content, nutrient uptake and metabolite profile. Significant root and shoot length improvement (112.65%, 53.70%); was recorded at treatment with *Aphanothece* sp and *C. ellipsoidea* CBEs respectively. Meanwhile, the highest root and shoot dry weight (DW) (34.81%, 58.69%) were obtained at treatment with *Aphanothece* sp. The latter also displayed the maximum uptake of



Nitrogen, phosphorus and potassium, which increased by 185.17%, 119.36% and 78.04% respectively compared with non-treated plants. Principal Component Analysis (PCA) confirmed that Phosphorus and Potassium levels in roots were closely related to enhanced Root length, whereas Nitrogen and chlorophyll b were closely related to Shoot and root DW. Additionally, Gas Chromatography-mass spectrometry (GC-MS) indicated that treatment with CBEs, induced the production of a vast array of metabolites. Treated plants recorded higher accumulation of palmitic and stearic acids, which could indicate a stimulation in de novo Lipid synthesis. CBEs also triggered the accumulation of pyridine-3-carboxamide (an amide active form of vitamin B3) and Linolenic acid; one of the key precursors in the biosynthetic pathway leading to plant jasmonates. Our results are a first step towards understanding the effects of microalgal extracts on plant physiology and biochemical pathways. Further investigations on biochemical fractionation of microalgal extracts and agronomic tests of their purified bioactive compounds could be a useful principal novelty for in-depth study of CBE action mechanisms. Other useful tools include; Comparative hormone profiling of treated and non-treated plants accompanied with combined High-Throughput Plant Phenotyping, transcriptomics and metabolomics analysis.

Upgrading industrial effluent for agricultural reuse: effects of digestate concentration and wood vinegar dosage on biosynthesis of plant growth promotor.

Environ Sci Pollut Res Int. 2020 Feb 11;;

Authors: Hosakul P, Kantachote D, Saritpongteeraka K, Phuttaro C, Chairapat S

Abstract

Emphasis on water reuse in agricultural sector receives a renewed interest to close the loop in circular economy, especially in dry and water-stressed regions. In this work, wastewater from cooperative smoked sheet rubber factory and the effluent (digestate) from its treatment system (anaerobic digester) were used as medium to grow purple non-sulfur bacteria (PNSB), *Rhodospseudomonas palustris* strain PP803, with wood vinegar supplement at mid-log growth phase to stimulate the release of 5-aminolevulinic acid (ALA), a plant growth promotor. Wastewater-to-digestate ratios (D:W) represented by soluble chemical oxygen demand (SCOD) were found to influence both the growth of *R. palustris* and synthesis of ALA. The highest ALA release of $16.02 \pm 0.75 \mu\text{M}$ and the biomass accumulation of $1302 \pm 78 \text{ mg/L}$ were obtained from the medium SCOD of 4953 mg/L. Although retarding biomass accumulation by 28-36%, wood vinegar (WV) addition was proven to improve ALA release by 40%. Result suggested that SCOD of 3438 mg/L (75:25 D:W) contained sufficient carbon source for PNSB growth and was chosen to subsequently run the photo-bioreactor (PBR) to sustain *R. palustris* PP803 cells production. In continuous PBR operation, PNSB proliferation suffered from the low organic concentration in PBR at low organic loading. An organic loading increase to 1.21 g COD/L day was found to attain highest biomass concentration and longest PNSB dominant period over microalgae. In this study, a real-time monitoring protocol of PNSB and microalgae was specifically developed based on image color analysis at acceptable accuracy ($R^2 = 0.94$). In the final assay, verification of the PBR-grown inoculant was conducted and ALA release efficiency was discussed under various wood vinegar dosages and dosing frequencies. This work has advanced our understandings closer to practical field application.



Natural pigments from microalgae grown in industrial wastewater.

Bioresour Technol. 2020 May;303:122894

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

Abstract

The aim of this study was to investigate the cultivation of *Nostoc* sp., *Arthrospira platensis* and *Porphyridium purpureum* in industrial wastewater to produce phycobiliproteins. Initially, light intensity and growth medium composition were optimized, indicating that light conditions influenced the phycobiliproteins production more than the medium composition. Conditions were then selected, according to biomass growth, nutrients removal and phycobiliproteins production, to cultivate these microalgae in food-industry wastewater. The three species could efficiently remove up to 98%, 94% and 100% of COD, inorganic nitrogen and PO_4^{3--P} , respectively. Phycocyanin, allophycocyanin and phycoerythrin were successfully extracted from the biomass reaching concentrations up to 103, 57 and 30 mg/g dry weight, respectively. Results highlight the potential use of microalgae for industrial wastewater treatment and related high-value phycobiliproteins recovery.

Simultaneous biohydrogen production from dark fermentation of duckweed and waste utilization for microalgal lipid production.

Bioresour Technol. 2020 Apr;302:122879

Authors: Mu D, Liu H, Lin W, Shukla P, Luo J

Abstract

A cost-effective and environmentally friendly method for biofuel production was developed, by utilizing duckweed as feedstock for biohydrogen production through dark fermentation and simultaneously using the fermentative waste to produce microalgal lipids. The results suggested that acid hydrolysis (1% H_2SO_4) was more suitable for the pretreatment of duckweed biomass. Maximum hydrogen production of 169.30 mL g⁻¹ dry weight was determined under a temperature of 35 °C and an initial pH of 7.0. After the dark fermentation, the volatile fatty acids (VFAs) including acetate and butyrate, were detected in the waste, with concentration determined as 1.04 g L⁻¹ and 1.52 g L⁻¹, respectively. During the mixotrophic cultivation of *Chlorella saccharophila* FACHB-4 using waste as feedstock, the maximum microalgal biomass and the lipid productions were about 2.8 and 33 times higher with respect to the autotrophic growth. The simultaneous biohydrogen production and waste utilization method provided a green strategy for biofuel production.



Characterization of a novel herbicide and antibiotic-resistant *Chlorella* sp. with an extensive extracellular matrix.

Photosynth Res. 2020 Mar;143(3):315-334

Authors: Nazos TT, Kokarakis EJ, Valsami EA, Stratigakis NC, Poloniataki EG, Sfindourakis GP, Ghanotakis DF

Abstract

A herbicide and antibiotic-resistant microalgal strain, isolated from a eutrophic site at Giofyros river (Heraklion, Crete, Greece) was extensively characterized. In the presence of relatively high concentrations of common photosynthesis inhibitors (DCMU and atrazine), as well as various antibiotics (spectinomycin, kanamycin, and chloramphenicol), the green microalga was able to increase its biomass in approximately equal levels compared to the control. Despite the high concentrations of the inhibitors, photosynthetic efficiency and chlorophyll a amount per dry cell biomass were comparable to those of control cultures in almost all cases. 18S rDNA analysis showed that this microalga belongs to the *Chlorella* genus. Optical and electron microscopy studies revealed the presence of an extensive extracellular matrix (EM) that surrounds the cells and plays an important role in colony formation and cell-cell interactions. Fourier transform infrared spectroscopy provided evidence that the EM consists of a polysaccharide. This matrix could be separated from the cells with a simple centrifugation. Depending on growth conditions, the dry cell biomass of this *Chlorella* strain was found to contain 35-39% proteins and 27-42% carbohydrates. The results of this study have demonstrated that the EM plays a protective role for cell homeostasis maintenance against the various chemical agents. This green microalga is a suitable candidate for further studies regarding sustainable biomass production in waste waters for a series of applications.

Microalgae as Functional Ingredients in Savory Food Products: Application to Wheat Crackers.

Foods. 2019 Nov 23;8(12):

Authors: Batista AP, Niccolai A, Bursic I, Sousa I, Raymundo A, Rodolfi L, Biondi N, Tredici MR

Abstract

Crackers are widely consumed snack foods and there is an increasing trend in adding functional ingredients to their composition. In the present work, the dried biomasses of four microalgae strains—*Arthrospira platensis* F&M-C256, *Chlorella vulgaris* Allma, *Tetraselmis suecica* F&M-M33, and *Phaeodactylum tricornutum* F&M-M40—were used as a source of proteins, antioxidants, and other bioactive molecules in artisanal wheat crackers. Two incorporation levels were tested: 2% (w/w) and 6% (w/w). The impact of microalgae addition was evaluated in terms of physical properties, biochemical composition, antioxidant activity, in vitro digestibility, and sensory characteristics. Microalgae crackers presented stable color and texture throughout eight weeks of storage. Microalgae crackers were slightly thinner and lighter than the control but presented a similar density in agreement with scanning electron microscope images, indicating that gas retention was not greatly affected by microalgae addition. Regarding biochemical composition,



6% *A. platensis* and *C. vulgaris* crackers presented a significantly higher protein content (13.2-13.5%), for which they could be claimed to be a "source of protein" according to the Regulation (EC) No. 1924/2006. *A. platensis* crackers showed the highest antioxidant activity and attained better sensory analysis scores. *T. suecica* and *P. tricornutum* crackers showed high phenolic content and antioxidant activity but attained low sensory scores mainly because of their unattractive fishy off-flavor.

Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows.

J Dairy Sci. 2019 Dec;102(12):10934-10946

Authors: Till BE, Huntington JA, Posri W, Early R, Taylor-Pickard J, Sinclair LA

Abstract

Modification of milk and cheese fat to contain long-chain n-3 fatty acids (FA) by feeding microalgae (ALG) to dairy cows has the potential to improve human health, but the subsequent effect on the sensory attributes of dairy products is unclear. The objective was to determine the effect of feeding dairy cows different amounts of ALG that was rich in docosahexaenoic acid (DHA) on milk and cheese FA profile, cheese sensory attributes, and cow performance. Twenty Holstein dairy cows were randomly allocated to 1 of 4 dietary treatments in a 4 × 4 row and column design, with 4 periods of 28 d, with cheddar cheese production and animal performance measurements undertaken during the final 7 d of each period. Cows were fed a basal diet that was supplemented with ALG (*Schizochytrium limacinum*) at 4 rates: 0 (control, C), 50 (LA), 100 (MA), or 150 g (HA) of ALG per cow per day. We found that both milk and cheese fat content of DHA increased linearly with ALG feed rate and was 0.29 g/100 g FA higher in milk and cheese from cows fed HA compared with C. Supplementation with ALG linearly reduced the content of saturated FA and the ratio of n-6:n-3 FA in milk and cheese. Supplementation with ALG altered 20 out of the 32 sensory attributes, with a linear increase in cheese air holes, nutty flavor, and dry mouth aftertaste with ALG inclusion. Creaminess of cheese decreased with ALG inclusion rate and was positively correlated with saturated FA content. We also observed a quadratic effect on fruity odor, which was highest in cheese from cows fed HA and lowest in LA, and firmness and crumbliness texture, being highest in MA and lowest in HA. Supplementation with ALG had no effect on the dry matter intake, milk yield, or live weight change of the cows, with mean values of 23.1, 38.5, and 0.34 kg/d respectively, but milk fat content decreased linearly, and energy-corrected milk yield tended to decrease linearly with rate of ALG inclusion (mean values of 39.6, 38.4, 37.1, and 35.9 g/kg, and 41.3, 41.3, 40.5, and 39.4 kg/d for C, LA, MA, and HA, respectively). We conclude that feeding ALG to high-yielding dairy cows improved milk and cheese content of DHA and altered cheese taste but not cow performance, although milk fat content reduced as inclusion rate increased.



Effects of multi-temperature regimes on cultivation of microalgae in municipal wastewater to simultaneously remove nutrients and produce biomass.

Appl Microbiol Biotechnol. 2019 Oct;103(19):8255-8265

Authors: Xu K, Zou X, Wen H, Xue Y, Qu Y, Li Y

Abstract

Coupling algal cultivation with wastewater treatment due to their potentials to alleviate energy crisis and reduce environmental burden has attracted the increased attention in recent years. However, these microalgal-based processes are challenging since daily and seasonal temperature fluctuation may affect microalgal growth in wastewater, and the effects of the temperature regimes on microalgal biomass production and wastewater nutrient removal remain unclear. In this study, *Chlorella vulgaris* was continuously cultured for 15 days in municipal wastewater to investigate the effects on the algal biomass and wastewater nutrient removal in three temperature regimes: (1) low temperature (4 °C), (2) high temperature (35 °C), and (3) alternating high-low temperature (35 °C in the day: 4 °C at night). Compared with the other two temperature regimes, the high-low temperature conditions generated the most biomass (1.62 g L⁻¹), the highest biomass production rate (99.21 mg L⁻¹ day⁻¹), and most efficient removal of COD, TN, NH₃-N, and TP (83.0%, 96.5%, 97.8%, and 99.2%, respectively). In addition, the polysaccharides, proteins, lipid content, and fatty acid methyl ester composition analysis indicates that in alternating high-low temperature condition, biomass production increased the potential for biofuel production, and there was the highest lipid content (26.4% of total dry biomass). The results showed that the nutrients except COD were all efficiently removed in these temperature conditions, and the alternating high-low temperature condition showed great potential to generate algal biomass and alleviate the wastewater nutrients. This study provides some valuable information for large-scale algal cultivation in wastewater and microalgal-based wastewater treatments.

Microalgal consortia for municipal wastewater treatment - Lipid augmentation and fatty acid profiling for biodiesel production.

J Photochem Photobiol B. 2020 Jan;202:111638

Authors: Sharma J, Kumar V, Kumar SS, Malyan SK, Mathimani T, Bishnoi NR, Pugazhendhi A

Abstract

The present study investigates the phycoremediation potentials of two microalgal consortia (MAC1 and MAC2) for treating sewage water and producing biomass with high lipid, protein and chlorophyll contents. During the study, the microalgal strains were tested for lipid enhancement, biomass production and contaminant removal from wastewater. The microalgal consortia showed prolific growth in wastewater with 75% dilution and accumulated higher lipid content of 31.33% dry cell weight in MAC1. The maximum biomass (50% diluted wastewater) for both the consortia was 1.53 and 1.04 gL⁻¹. Total chlorophyll (19.17-25.17 µg mL⁻¹) and protein contents (0.12-0.16 mg mL⁻¹) for both the consortia were found to be maximum in 75 WW. MAC1 was



capable of removing 86.27% of total organic carbon and 87.6% of chemical oxygen demand. Approximately, 94% of nitrate and phosphate contents were removed from the initial contents of wastewater. Heavy metal removal efficiency was also found to be better and showed 85.06% Cu, 75.2% Cr, 98.2% Pb, and 99.6% Cd removal by the algal consortia. Pyrolytic decomposition of algal consortia was observed using thermogravimetric analysis. The stepwise decomposition of algae indicated distinct losses of functional groups. The gas chromatography-mass spectrometric analysis revealed the majority of saturated fatty acids followed by monounsaturated and polyunsaturated fatty acids. Thus, the present study proved that both the consortia show tremendous potential for the treatment of domestic wastewaters with successive lipid enhancement for biodiesel production.

Biogas yields and composition from oil-extracted halophilic algae residues in conventional biogas plants operated at high salinities.

Bioprocess Biosyst Eng. 2019 Dec;42(12):1915-1922

Authors: Adamietz T, Jurkowski W, Adolph J, Brück TB

Abstract

CO₂-induced climate change drives the development of renewable processes for industrial products. Algae processes can actively fix and convert CO₂ into value adding products, such as oils. Algae lipids hence counteract climate change and provide access to renewable commodities. In this context, valorization of algal residues remaining after oil extraction is a challenge for the emerging cyclic bioeconomy. This study focuses on the valorization of oil-extracted algae residues derived from the halophilic strain *Scenedesmus obliquus* via anaerobic digestion. We examined the effect of prior oil extraction on microbial digestibility and increasing salt content in the substrate with regard to biogas yield and composition. Our cumulative data demonstrate that the supercritical CO₂ oil extraction acts as a physical pretreatment that facilitates enhanced hydrolysis of both polymeric cell wall carbohydrates and cellular proteins, providing methane yields of 213.2 LN kg⁻¹ VS day⁻¹. Methane yields were 20% higher than literature values obtained with the same algae strain in the absence of prior oil extraction. We obtained these superior results albeit all lipids and nonpolar proteins had been extracted from the biogas substrate. Our data indicate that continuous anaerobic digestion without loss of fermentation efficiency is feasible up to a salt concentration of 2% w/v, if conventional, agricultural biogas plants are gradually adapted to the salt content of the substrate. Monofermentation of the investigated oil-extracted algae residue is technically feasible at loading rates of 1.5 kg VS m⁻³ day⁻¹, but a supplementation with carbohydrate rich biomass would prove beneficial to alleviate ammonia inhibition.



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, Prosin 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 be recovered by using traditional extraction procedures.

Selective Extraction of ω -3 Fatty Acids from *Nannochloropsis* sp. Using Supercritical CO₂ Extraction.

Molecules. 2019 Jun 29;24(13):

Authors: Leone GP, Balducci R, Mehariya S, Martino M, Larocca V, Di Sanzo G, Iovine A, Casella P, Marino T, Karatza D, Chianese S, Musmarra D, Molino A

Abstract

In this article, microalgae *Nannochloropsis* sp. was used for fatty acid (FA) extraction, using a supercritical fluid-carbon dioxide (SF-CO₂) extraction method. This study investigated the influence of different pre-treatment conditions by varying the grinding speed (200-600 rpm), pre-treatment time (2.5-10 min), and mixing ratio of diatomaceous earth (DE) and *Nannochloropsis* sp. biomass (0.5-2.0 DE/biomass) on FAs extraction. In addition, the effect of different operating conditions, such as pressure (100-550 bar), temperature (50-75 °C), and CO₂ flow rate (7.24 and 14.48 g/min) on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) recovery, was analyzed. Experimental data evidenced that, keeping constant the extraction conditions, the pre-treatment step enhanced the FAs extraction yield up to 3.4 fold, thereby the maximum extracted amount of FAs (61.19 mg/g) was attained with the pre-treatment with a ratio of DE/biomass of 1 at 600 rpm for 5 min. Moreover, by increasing both SF-CO₂ pressure and temperature, the selectivity towards EPA was enhanced, while



intermediate pressure and lower pressure promoted DHA recovery. The highest amount of extracted EPA, i.e., 5.69 mg/g, corresponding to 15.59%, was obtained at 75 °C and 550 bar with a CO₂ flow rate of 14.48 g/min, while the maximum amount of extracted DHA, i.e., ~0.12 mg/g, equal to 79.63%, was registered at 50 °C and 400 bar with a CO₂ flow rate of 14.48 g/min. Moreover, the increased CO₂ flow rate from 7.24 to 14.48 g/min enhanced both EPA and DHA recovery.

Use of ultrasound for aiding lipid extraction and biodiesel production of microalgae harvested by chitosan.

Environ Technol. 2020 Apr 14;:1-8

Authors: Ma G, Mu R, Capareda SC, Qi F

Abstract

In this work, chitosan, a biodegradable flocculant, was investigated to determine its utility in flocculating microalgae, its effect on cell integrity, and its impact on lipid extraction and the conversion to fatty acid methyl ester (FAME). Results showed that chitosan adequately performed flocculation on *Chlorella vulgaris* microalgae and achieved a high harvesting efficiency of $96.35 \pm 1.96\%$ when implemented under the following conditions: chitosan dose = 120 mg/L⁻¹, pH = 5, mixing speed = 150 rpm for 20 min, followed by 10 min of settling time. Moreover, scanning electron microscope (SEM) combined with transmission electron microscope (TEM) demonstrated that chitosan protected the cells' structure from morphological damage. Finally, the highest lipid extraction yield and biodiesel production was obtained from the chitosan-harvested biomass when the microalgae were pretreated with ultrasound.

Integrated ultrasound-assisted liquid biphasic flotation for efficient extraction of astaxanthin from *Haematococcus pluvialis*.

Ultrason Sonochem. 2020 Mar 04;67:105052

Authors: Khoo KS, Chew KW, Yew GY, Manickam S, Ooi CW, Show PL

Abstract

The purpose of this investigation is to evaluate the implementation of ultrasound-assisted liquid biphasic flotation (LBF) system for the recovery of natural astaxanthin from *Haematococcus pluvialis* microalgae. Various operating conditions of ultrasound-assisted LBF systems such as the position of ultrasound horn, mode of ultrasonication (pulse and continuous), amplitude of ultrasonication, air flowrate, duration of air flotation, and mass of *H. pluvialis* microalgae were evaluated. The effect of ultrasonication on the cellular morphology of microalgae was also assessed using microscopic analysis. Under the optimized operating conditions of UALBF, the maximum recovery yield, extraction efficiency, and partition coefficient of astaxanthin were $95.08 \pm 3.02\%$, $99.74 \pm 0.05\%$, and 185.09 ± 4.78 , respectively. In addition, the successful



scale-up operation of ultrasound-assisted LBF system verified the practicability of this integrated approach for an effective extraction of natural astaxanthin.

Optimization of Ultrasound-Assisted Extraction Condition for Phenolic Compounds, Antioxidant Activity, and Epigallocatechin Gallate in Lipid-Extracted Microalgae.

Molecules. 2020 Jan 21;25(3):

Authors: Gam DH, Yi Kim S, Kim JW

Abstract

Lipid-extracted microalgae (LEM, Tetraselmis KCTC 12236BP), a solid waste by-product obtained from algal biodiesel production, is typically considered a rich source of antioxidant compounds, including phenolic compounds. The purpose of this study was to apply a statistically-based methodology to enhance the extraction of total phenolic compounds (TPCs) and antioxidant activity (AA) from LEM and to verify the production of epigallocatechin gallate (EGCG), a bioactive material, under optimum conditions. The optimal extractions of TPC and AA were explored by varying the key variables, including the extraction temperature, ethanol concentration, extraction time, and ultrasonic power, through statistical optimization. The optimal extraction conditions were identified through 27 runs following the central composite design. The regression analyses of TPC and AA showed good fit of the experimental data to the second-order polynomial models, with coefficient of determination (R^2) values of 0.8769 and 0.8432, respectively. In the variation experiment, the maximum TPC and AA values of 9.8 mg GAE/g and 91.8% were obtained respectively with an extraction temperature of 74.4 °C, ethanol concentration of 55.4%, extraction time of 59.6 min, and ultrasonic power of 700 W. HPLC coupled with diode array detection was used to identify and quantify the phenolic compounds in the extracts, and EGCG (0.12 mg/g DM) was identified as a major peak in the analysis, demonstrating that high value-added material with a bioactive property can be produced from LEM. The results indicated that statistical optimization is applicable for optimizing the extraction of TPC and AA from LEM and provided a scientific basis for applying ultrasound-assisted extraction on an industrial scale by optimizing the conditions. LEM has a high TPC value, particularly with regard to EGCG, and excellent AA, considering it is highly used as a functional material for food, cosmetics, and medicine.

Intensified recovery of lipids, proteins, and carbohydrates from wastewater-grown microalgae *Desmodesmus* sp. by using ultrasound or ozone.

Ultrason Sonochem. 2020 Apr;62:104852

Authors: González-Balderas RM, Velásquez-Orta SB, Valdez-Vazquez I, Orta Ledesma MT



Abstract

This study evaluates the effect of ultrasound and ozone pretreatments for the subsequent recovery of *Desmodemus* sp. biocomponents-lipids, proteins, and carbohydrates-using a response surface methodology. Both pretreatments impact on the recovered lipids quality, solvent waste production and extraction time is analysed for process intensification purposes. For ultrasound pretreatment, independent parameters were energy applied (50-200 kWh/kg dry biomass), biomass concentration (25-75 g/L), and ultrasonic intensity (0.32 and 0.53 W/mL). While for ozone pretreatment, independent parameters were ozone concentration (3-9 mg O₃/L), biomass concentration (25-75 g/L), and contact time (5-15 min). In the case of ultrasound pretreatment, recovery yield reached $97 \pm 0.4\%$, $89 \pm 3\%$, and $73 \pm 0.6\%$ for proteins, carbohydrates and lipids respectively. Given process required: energy applied of 50 kWh/kg dry biomass, 75 g/L of biomass concentration, 0.32 W/mL of ultrasonic intensity, and 56 min of time process. Ultrasound caused high cell disruption releasing all proteins, thereby obviating downstream processing for its recovery. Ozone pretreatment recovery yield was $85 \pm 2\%$, $48 \pm 1.4\%$, and $25 \pm 1.3\%$, for carbohydrates, lipids and proteins respectively, under the following conditions: 9 mg O₃/L of ozone concentration, 25 g/L of biomass concentration, and 5 min of contact time that depicts an energy consumption of 30.64 kWh/kg dry biomass. It was found that ultrasound and ozone pretreatments intensified the lysis and biocomponents recovery process by reducing solvent consumption by at least 92% and extraction time between 80% and 90% compared with extraction of untreated biomass biocomponents. Both pretreatments improve the composition of the recovered lipids. It was noted that the yield of neutral lipids increased from 28% to 67% for ultrasound pretreatment while for ozone pretreatment from 49% to 63%. The method used for lipid extraction may also have an effect but here it was kept constant.

Wastewater microalgal production, nutrient removal and physiological adaptation in response to changes in mixing frequency.

Water Res. 2014 Sep 15;61:130-40

Authors: Sutherland DL, Turnbull MH, Broady PA, Craggs RJ

Abstract

Laminar flows are a common problem in high rate algal ponds (HRAP) due to their long channels and gentle mixing by a single paddlewheel. Sustained laminar flows may modify the amount of light microalgal cells are exposed to, increase the boundary layer between the cell and the environment and increase settling out of cells onto the pond bottom. To date, there has been little focus on the effects of the time between mixing events (frequency of mixing) on the performance of microalgae in wastewater treatment HRAPs. This paper investigates the performance of three morphologically distinct microalgae in wastewater treatment high rate algal mesocosms operated at four different mixing frequencies (continuous, mixed every 45 min, mixed every 90 min and no mixing). Microalgal performance was measured in terms of biomass concentration, nutrient removal efficiency, light utilisation and photosynthetic performance. Microalgal biomass increased significantly with increasing mixing frequency for the two colonial species but did not differ for the single celled species. All three species were more efficient at NH₄-N uptake as the frequency of mixing increased. Increased frequency of mixing



supported larger colonies with improved harvest-ability by gravity but at the expense of efficient light absorption and maximum rate of photosynthesis. However, maximum quantum yield was highest in the continuously mixed cultures due to higher efficiency of photosynthesis under light limited conditions. Based on these results, higher microalgal productivity, improved wastewater treatment and better gravity based harvest-ability can be achieved with the inclusion of more mixing points and reduced laminar flows in full-scale HRAP.

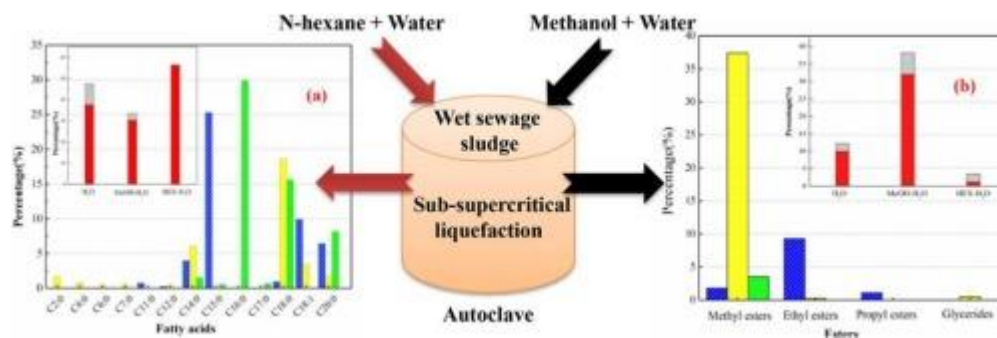
Sub-supercritical liquefaction of municipal wet sewage sludge to produce bio-oil: Effect of different organic–water mixed solvents

Source:The Journal of Supercritical Fluids, Volume 138

Author(s): Rundong Li, Zhiming Ma, Tianhua Yang, Bingshuo Li, Lihong Wei, Yang Sun

The effect of different mixed solvents (methanol–water (MeOH–H₂O), n-hexane–water (HEX–H₂O)) on liquefaction of municipal wet sewage sludge (WSS) was investigated in this study. The highest bio-oil yield of 46.5 wt% was achieved in MeOH–H₂O, of which ester content was 38.1% and methyl ester comprised 37.4%. The lowest solid residue yield of 10.3 wt% was achieved in HEX–H₂O. The highest fatty acid compounds content was 54.9% of bio-oil obtained in HEX–H₂O, of which were in saturated state. The highest calorific value and the lowest oxygen content of bio-oil were achieved in HEX–H₂O: 36.45 ± 0.38 MJ/kg and 7.65 ± 0.26 wt%, respectively. The thermal stability of solid residue was enhanced in HEX–H₂O. The formation of esters was promoted in MeOH–H₂O, whereas aliphatic compounds could be efficiently extracted in HEX–H₂O.

Graphical abstract



Bioaugmentation strategy for enhancing anaerobic digestion of high C/N ratio feedstock with methanogenic enrichment culture

Source:Bioresour. Technol., Volume 261

Author(s): Ying Li, Lianhua Li, Yongming Sun, Zhenhong Yuan

To investigate whether bioaugmentation could improve the digestion performance of high C/N ratio feedstock without co-digestion with nitrogen-rich substrate, different forms of enriched methanogenic culture were introduced to the continuous feed digesters. The performance efficiency of bioaugmentation on digestion improvement was compared. The effect of bioaugmentation on microbial community composition was revealed as well. Results demonstrated that routine bioaugmentation with liquid culture (containing the microbes and the medium remains) showed the best performance, with the organic loading rate (OLR), methane percentage, volumetric methane production (VMP) and volatile solid methane production (VSMP) higher at $1.0 \text{ g L}^{-1} \text{ d}^{-1}$, 24%, $0.22 \text{ LL}^{-1} \text{ d}^{-1}$ and $0.23 \text{ Lg}^{-1} \text{ VS d}^{-1}$ respectively, compared to the non-bioaugmentation control. Whole genome pyrosequencing analysis suggested that consecutive microbial consortium addition could reconstruct the methanogens community by increasing the populations of acetoclastic methanogens *Methanotherix*, which could accelerate the degradation of acetate and methane production.

Preparation of bio-bitumen by bio-oil based on free radical polymerization and production process optimization

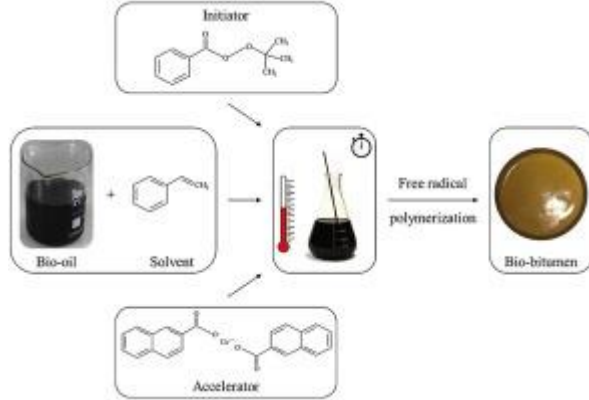
Source: Journal of Cleaner Production, Volume 189

Author(s): Zhaojie Sun, Junyan Yi, Decheng Feng, Cor Kasbergen, Athanasios Scarpas, Yiming Zhu

Bio-oil produced during the production of biodiesel is a burden to the environment. Recycling and utilization of bio-oil as a substitute for pavement bitumen can help to build an environmentally-friendly and clean infrastructure. In this study, the bio-bitumen was prepared by bio-oil based on free radical polymerization. Different kinds of bio-bitumen products were produced by reacting bio-oil with an initiator and an accelerator solution at different reaction conditions. The orthogonal experimental method was employed to determine the optimal bio-bitumen production process by evaluating the indices of viscosity, rutting factors and fatigue factors. The test results show that the optimal mass proportions of bio-oil:initiator:accelerator solution is 100:1:2. Materials with these mass proportions should react at $100 \text{ }^\circ\text{C}$ for 2 h to yield the best bio-bitumen product. This kind of bio-bitumen product can be considered as a promising substitute for traditional petroleum bitumen.

Graphical abstract





Chloroplast engineering of *Chlamydomonas reinhardtii* to use phosphite as phosphorus source

Source: Algal Research, Volume 33

Author(s): José M. Sandoval-Vargas, Karla S. Macedo-Osorio, Noé V. Durán-Figueroa, Claudio Garibay-Orijel, Jesús A. Badillo-Corona

Phosphorus (P) is a key biological element and a limiting nutrient in aquatic and terrestrial environments. The vast majority of organisms can only uptake phosphorus in its most oxidized form, as phosphate (PO_4^{3-}), whereas a few prokaryotic species can metabolize phosphorus in a more reduced state such as phosphite (PO_3^{3-}). Recently, it has been shown that by expressing the *ptxD* gene, encoding a NAD-dependent phosphite dehydrogenase (also known as phosphonate dehydrogenase), in *Chlamydomonas reinhardtii* phosphite utilization can be enabled. However, this was done by transforming the nuclear genome, where gene silencing is frequent and random integration of transgenes can result in variable levels of gene expression and pleiotropic effects. The aim of this work was to investigate if phosphite assimilation in the eukaryotic algae *Chlamydomonas reinhardtii* can also be achieved by expressing a codon-optimized *ptxD* gene in the chloroplast. To do this, the *ptxD* gene was targeted and stably integrated into the *psbA* exon 5-5S rRNA intergenic region within the inverted repeats of the *C. reinhardtii* chloroplast genome. Integration was shown to occur in the targeted site and transplastomic lines were shown to be homoplasmic and to stably accumulate the NAD-dependent phosphite dehydrogenase PTXD, enabling the cells to use phosphite as the sole phosphorus source in a 0.1–5 mM concentration range. This work demonstrates that transplastomic lines of *C. reinhardtii* expressing the prokaryotic *ptxD* gene can effectively be cultivated in phosphite, opening new opportunities for microalgae cultivation.



Impact of thiamine metabolites and spent medium from *Chlorella sorokiniana* on metabolism in the green algae *Auxenochlorella protothecoides*

Source:Algal Research, Volume 33

Author(s): Brendan T. Higgins, Qichen Wang, Sandon Du, Marie Hennebelle, Ameer Y. Taha, Oliver Fiehn, Jean S. VanderGheynst

Auxenochlorella protothecoides is a known thiamine auxotroph but our past work has shown that it can synthesize thiamine if provided with the precursor molecule 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP). Partial thiamine auxotrophy is common in microalgae with important ramifications for global phytoplankton productivity as well as engineering applications of algae. While thiamine deficiency can greatly depress algae growth and lipid content, the detailed metabolic impacts of thiamine deficiency are not well understood. We used metabolomics to study the response to thiamine-limited and replete conditions in mixotrophic *A. protothecoides*. We also investigated the impacts of exogenous HMP addition and the use of spent medium from another green algae, *C. sorokiniana*, as a source of thiamine metabolites. This is the first study, to our knowledge, that addresses metabolic impacts of thiamine deficiency and alleviation in green microalgae. Thiamine deficient cultures exhibited accumulation of pyruvate and α -ketoglutarate, indicating bottlenecks at the pyruvate dehydrogenase (PDH) and oxoglutarate dehydrogenase (OGDH) complexes. Both PDH and OGDH require thiamine pyrophosphate (TPP) as a cofactor. Transketolase also requires TPP but we only observed build-up of ribose-5-phosphate when glucose was supplied as a substrate. As expected, thiamine and HMP addition could alleviate these metabolic bottlenecks while greatly increasing algal growth, neutral lipid and starch content. Spent medium from *C. sorokiniana* only appeared to partially alleviate thiamine deficiency and resulted in build-up of isocitrate and glycolate, metabolites that appeared relatively unaffected by the presence or absence of thiamine. Interestingly, longer culture time of *C. sorokiniana* when preparing the spent medium led to much higher availability of thiamine metabolites. Thus, under the right conditions, it may be possible to co-culture mutually beneficial algae species and/or recycle spent cultivation medium to overcome auxotrophy in algae.

Exergy efficiency of solar energy conversion to biomass of green macroalgae *Ulva* (Chlorophyta) in the photobioreactor

Source:Energy Conversion and Management, Volume 167

Author(s): Meiron Zollmann, Hadar Traugott, Alexander Chemodanov, Alexander Liberzon, Alexander Golberg

Offshore production of macroalgae biomass, which was recently given the name seagriculture, is one of the important but least explored alternative energy resources. Unlike microalgae, macroalgae cultivation can be done offshore and therefore brings real news to the biofuel – food

land agriculture conflict. A wide variety of small-scale laboratory experiments are done lately in order to deepen the knowledge and develop expertise in macroalgae cultivation and its downstream processing. For energy applications, it is common to evaluate the performance of an energy source or system in exergy efficiency terms. Another important parameter that is evaluated to determine the system's environmental impact is its volumetric and areal footprint. The current work examines two exergy efficiency indexes, the Exergy Efficiency (EE), which takes into account all exergy inputs, and the Exergy Return On Investment (ExROI), that includes only fossil fuel exergy inputs, both on a green macroalgae *Ulva* grown in the macroalgae photobioreactor system (MPBR) incorporated into a building. Cultivation of macroalgae in the building embedded MPBR achieved maximal values of 0.012 and 0.22 for EE and ExROI, compared to a range of 0.05–8.34 and 0.013–0.327 found in published papers of microalgae systems. In addition, a modelled optimization of the initial biomass density leads to maximal values of about 0.035 for EE and 0.433 for ExROI, while further improvement may be achieved by optimization of nutrient addition and mixing methodology. This work demonstrates a tool to measure the performance of laboratory scale macroalgae biomass cultivation systems, followed by preliminary efficiency and environmental impact values, important for future upscaling.

Optimising light conditions increases recombinant protein production in *Chlamydomonas reinhardtii* chloroplasts

Source: Algal Research, Volume 32

Author(s): Saskya E. Carrera Pacheco, Ben Hankamer, Melanie Oey

The green alga *Chlamydomonas reinhardtii* provides a platform for cheap, scalable and safe production of complex proteins. Despite the fact that chloroplast gene expression in photosynthetic organisms is tightly regulated by light, most expression studies have analysed chloroplast recombinant protein production under constant light. Here, the influence of light period and intensity on expression of green fluorescent protein (GFP) and a GFP-bacterial-lysin (PlyGBS) fusion protein was analysed. Protein yields were strongly influenced by the light period (6–24 h d⁻¹), the light intensity (0–450 μE m⁻² s⁻¹) and trophic condition. Heterotrophic conditions showed low yields of both recombinant proteins due to low growth rates, despite high protein accumulation per cell. Mixotrophic conditions exhibited the highest yields for GFP (4 mg·L⁻¹·d⁻¹) under constant light at 35 μE m⁻² s⁻¹ and GFP-PlyGBS (0.4 mg·L⁻¹·d⁻¹) under a light period of 15 h d⁻¹ and 35 μE m⁻² s⁻¹. This is due to the high growth rates and cellular protein content. For GFP-PlyGBS the maximum increase in cellular protein accumulation was ~24-fold, and in total protein yield ~10-fold, in comparison to constant light conditions (~200 μE m⁻² s⁻¹). The highest yields under photoautotrophic conditions were obtained under a 9 h d⁻¹ light period. GFP yielded 1.2 mg·L⁻¹·d⁻¹ and GFP-PlyGBS 0.42 mg·L⁻¹·d⁻¹. This represented a ~5-fold increase in cellular protein accumulation for GFP-PlyGBS in comparison to constant light conditions (~200 μE m⁻² s⁻¹). Optimising light conditions to balance growth and protein expression can significantly enhance overall recombinant protein production in *C. reinhardtii* cultures.



Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns

Source:Biotechnology Advances, Volume 36, Issue 4

Author(s): Giorgos Markou, Liang Wang, Jianfeng Ye, Adrian Unc

Aquatic organisms, such as microalgae (*Chlorella*, *Arthrospira* (*Spirulina*), *Tetraselmis*, *Dunaliella* etc.) and duckweed (*Lemna* spp., *Wolffia* spp. etc.) are a potential source for the production of protein-rich biomass and for numerous other high-value compounds (fatty acids, pigments, vitamins etc.). Their cultivation using agro-industrial wastes and wastewater (WaW) is of particular interest in the context of a circular economy, not only for recycling valuable nutrients but also for reducing the requirements for fresh water for the production of biomass. Recovery and recycling of nutrients is an unavoidable long-term approach for securing future food and feed production. Agro-industrial WaW are rich in nutrients and have been widely considered as a potential nutrient source for the cultivation of microalgae/duckweed. However, they commonly contain various hazardous contaminants, which could potentially taint the produced biomass, raising various concerns about the safety of their consumption. Herein, an overview of the most important contaminants, including heavy metals and metalloids, pathogens (bacteria, viruses, parasites etc.), and xenobiotics (hormones, antibiotics, parasiticides etc.) is given. It is concluded that pretreatment and processing of WaW is a requisite step for the removal of several contaminants. Among the various technologies, anaerobic digestion (AD) is widely used in practice and offers a technologically mature approach for WaW treatment. During AD, various organic and biological contaminants are significantly removed. Further removal of contaminants could be achieved by post-treatment and processing of digestates (solid/liquid separation, dilution etc.) to further decrease the concentration of contaminants. Moreover, during cultivation an additional removal may occur through various mechanisms, such as precipitation, degradation, and biotransformation. Since many jurisdictions regulate the presence of various contaminants in feed or food setting strict safety monitoring processes, it would be of particular interest to initiate a multi-disciplinary discussion whether agro-industrial WaW ought to be used to cultivate microalgae/duckweed for feed or food production and identify most feasible options for doing this safely. Based on the current body of knowledge it is estimated that AD and post-treatment of WaW can lower significantly the risks associated with heavy metals and pathogens, but it is yet unclear to what extent this is the case for certain persistent xenobiotics.

Performance and mechanism of a novel algal-bacterial symbiosis system based on sequencing batch suspended biofilm reactor treating domestic wastewater

Source:Bioresource Technology, Volume 265

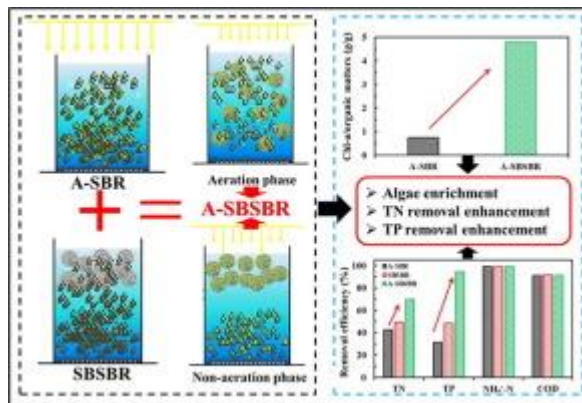
Author(s): Cong-Cong Tang, Yu Tian, Zhang-Wei He, Wei Zuo, Jun Zhang

A novel algal-bacterial symbiosis system based on sequencing batch suspended biofilm reactor



(A-SBSBR) was developed for simultaneously enhanced nitrogen (N) and phosphorus (P) removal from domestic wastewater. Results showed that the total N (TN) and P (TP) removal efficiencies in A-SBSBR increased to 69.91% and 94.78%, respectively. The mechanism analysis indicated that TN removal mainly occurred at non-aeration stage, and TP removal happened during the whole cycle in A-SBSBR. Compared to control SBSBR, TN removal by denitrification and anabolism and TP removal by anabolism in A-SBSBR increased by 12.70%, 7.64% and 50.13%, respectively. The Chlorophyll a accumulation in biofilm increased to 4.80 ± 0.08 mg/g. Algae related to *Chlorella* and *Scenedesmus* and bacteria related to *Flavobacterium*, *Micropruina* and *Comamonadaceae* were enriched in A-SBSBR and responsible for the enhanced nutrients removal effect. This study may provide a new solution to achieve nutrients removal enhancement from wastewater.

Graphical abstract



Enhancement of Spirulina biomass production and cadmium biosorption using combined static magnetic field

Source: Bioresource

Technology,

Volume

265

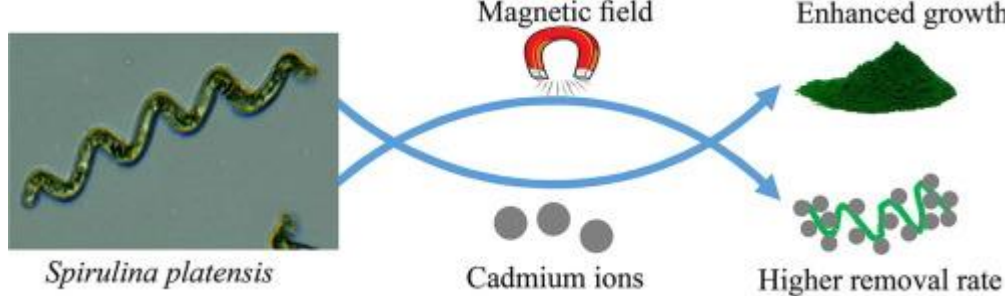
Author(s): Weilan Shao, Reham Ebaid, Abd El-Fatah Abomohra, Mohamed Shahen

The effect of static magnetic field (SMF) on *Spirulina platensis* growth and its influence on cadmium ions (Cd^{2+}) removal efficiency were studied. Application of 6 h day^{-1} SMF resulted in the highest significant biomass productivity of $0.198 \text{ g L}^{-1} \text{ day}^{-1}$. However, 10 and 15 mg L^{-1} of Cd^{2+} resulted in significant reduction in biomass productivity by 8.8 and 12.5%, respectively, below the control. Combined SMF showed 30.1% significant increase in biomass productivity over the control. On the other hand, increase of initial Cd^{2+} concentration resulted in significant reduction of Cd^{2+} removal efficiency, representing 79.7% and 61.5% at 10 and 15 mg L^{-1} , respectively, after 16 days. Interestingly, application of SMF for 6 h day^{-1} enhanced Cd^{2+} removal efficiency counted by 91.4% and 82.3% after 20 days for cultures with initial Cd^{2+} concentration of 10 and 15 mg L^{-1} , representing increase by 6.3 and 25.3%, respectively, over the SMF-untreated cultures.

Graphical abstract



Ministerio de Agricultura,
Ganadería y Pesca
Argentina



Integrated microalgae biomass production and olive mill wastewater biodegradation: Optimization of the wastewater supply strategy

Source: Chemical Engineering Journal, Volume 349

Author(s): Fabrizio Di Caprio, Pietro Altimari, Francesca Pagnanelli

Olive mill wastewater (OMW) was supplied to *Scenedesmus* sp. cultures to simultaneously achieve biomass production and wastewater biodegradation. Two OMW supply strategies were implemented to prevent the reduced growth performances that are attained, compared to photoautotrophic cultivation, when OMW is supplied at the beginning of cultivation (batch strategy). A fed-batch strategy including the gradual OMW supply yielded a biomass production equal to 0.86 g/L, while 1.4 g/L was attained by a two-stage strategy including OMW addition during nitrogen-starvation. OMW enhanced the carbohydrate accumulation (up to 44%) through the removal of OMW sugars (60–70%). About 55% OMW phenol removal was achieved by the fed-batch strategy when the phenol concentration was lower than 100 mg/L, and by the two-stage strategy when the heterotrophic stage lasted longer than 8–10 days. The illustrated results indicate that the OMW supply strategy can be purposefully tailored to regulate biomass production and OMW biodegradation.

Sugarcane bagasse as a novel carbon source for heterotrophic cultivation of oleaginous microalga *Schizochytrium* sp.

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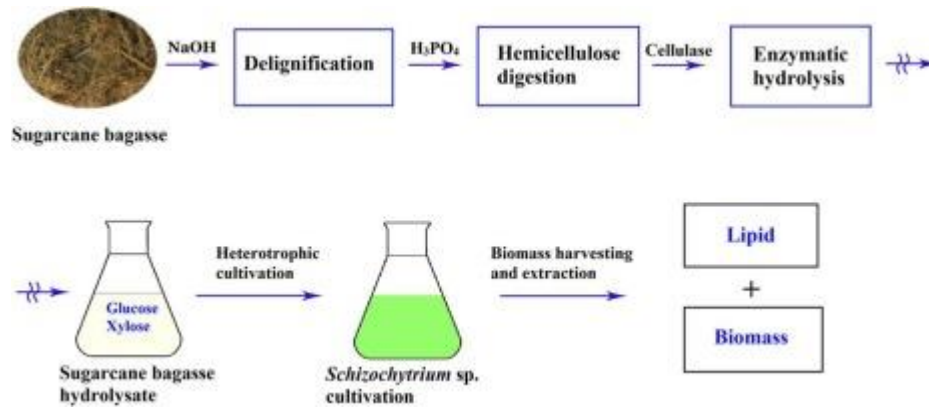
Author(s): Hoang Chinh Nguyen, Chia-Hung Su, Yuan-Kun Yu, Dinh Thi My Huong

Oleaginous microalgae are known as promising oil producers, which accumulate high amount of lipids from various carbon substrates. This study first investigated sugarcane bagasse hydrolysate as a cheap carbon source for the production of biomass and lipid by *Schizochytrium* sp. The sugarcane bagasse was pretreated with alkali followed by phosphoric acid to remove lignin and enhance xylose production, respectively. The enzymatic hydrolysis of the pretreated sugarcane bagasse by cellulase was subsequently optimized. A maximum glucose yield of 95.77% was obtained at an enzyme loading of 0.3 mL/g with a hydrolysis reaction time of 48 h.



The sugarcane bagasse hydrolysate containing glucose and xylose was subsequently used as a substrate for cultivating *Schizochytrium* sp. Results showed that sugarcane bagasse hydrolysate performed better than refined glucose for cell growth and lipid accumulation. The maximum biomass (10.45 g/L) and lipid content (45.15%) were achieved by growing *Schizochytrium* sp. in a medium containing 40 g/L glucose in sugarcane bagasse hydrolysate for 72 h. Sugarcane bagasse hydrolysate also resulted in higher levels of polyunsaturated fatty acid and docosahexaenoic acid than did refined glucose. This study suggests that sugarcane bagasse hydrolysate is a low-cost and effective carbon source for microalgal biomass and lipid production.

Graphical abstract



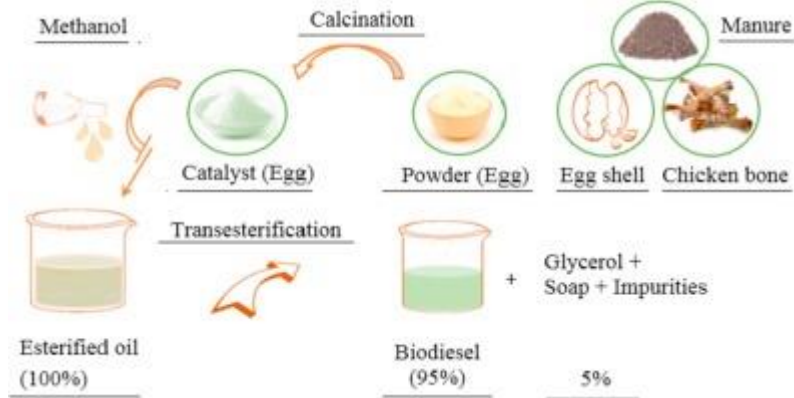
Valorization of harmful algae *E. compressa* for biodiesel production in presence of chicken waste derived catalyst

Source: Renewable Energy, Volume 129, Part A

Author(s): M.A. Rahman

The main objective of this investigation is to compare catalytic activity of three different catalysts derived from chicken waste. The prepared catalysts were characterized by DTG, SEM, BET, XRD, and FT-IR. The effect of catalysts (chicken manure, chicken bone, and egg shell) on biodiesel yield (from *E. compressa* algal oil) were evaluated by varying calcination temperature, reaction temperature, molar ratio, catalyst amount, mixing speed, reaction time, and water content in methanol. The maximum biodiesel yield was obtained for egg shell catalyst-95% while for chicken bone catalyst -94% and chicken manure catalyst -85% at 9:1 M, 5% (w/w) of catalyst amount, 65 °C, 2% water content, and 400 rpm for 3 h. The chicken bone catalyst is highly active in reaction but in presence of water content (max. 2%) in methanol egg shell shows higher biodiesel yield performance. The recovered catalyst can be used maximum three times with a slight deviation. The quality of biodiesel merged with standards value. The conversion of waste raw materials into valuable resources make this method a cost-effective and reveals a recyclable procedure for the production of biodiesel.

Graphical abstract



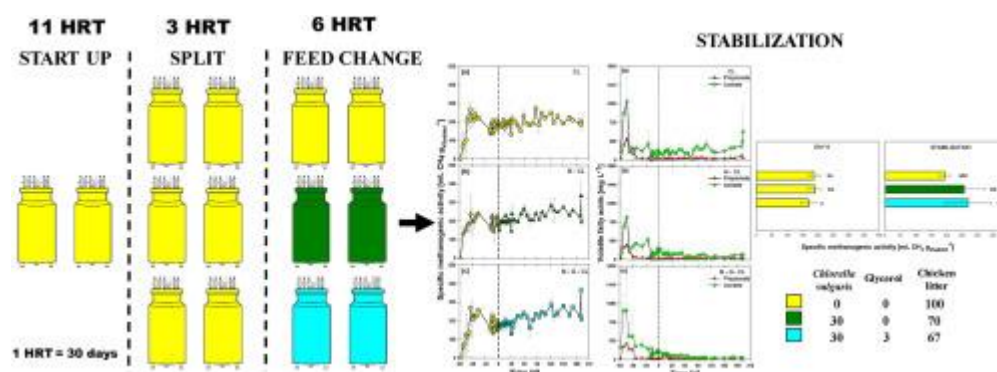
Oil-extracted *Chlorella vulgaris* biomass and glycerol bioconversion to methane via continuous anaerobic co-digestion with chicken litter

Source: Renewable Energy, Volume 128, Part A

Author(s): José Carlos Meneses-Reyes, Guadalupe Hernández-Eugenio, David H. Huber, Nagamani Balagurusamy, Teodoro Espinosa-Solares

The objective of this work was to evaluate the performance of mesophilic continuous anaerobic co-digestion using oil-extracted microalgae (M) and glycerol (G) in co-digestion with chicken litter (CL). The process included the starting up and stabilization of continuous anaerobic bioreactors using CL as a feedstock and the corresponding adaptation to M-CL and M-G-CL feedstocks. The treatments were selected based on a previous report of our research team on Biochemical Methane Potential (BMP) evaluation, taking in consideration only the best M-G-CL feedstock ratios. The performance was evaluated by the Specific Methanogenic Activity (SMA); the best response (270.0 mL CH₄ gVS added⁻¹) was obtained with a triple co-digestion M-G-CL 30:3:67, which was 39.0% above the CL treatment. These findings have shown that the two main residuals from microalgae biodiesel production (G and M) can be used as a feedstock to improve methane production through anaerobic digestion.

Graphical abstract



Southern Australian seaweeds: A promising resource for omega-3 fatty acids

Source:Food Chemistry, Volume 265

Author(s): Matthias Schmid, Lesleigh G.K. Kraft, Luna M. van der Loos, Gerald T. Kraft, Patti Virtue, Peter D. Nichols, Catriona L. Hurd

To assess the suitability of southern-Australian macroalgae as potential marine resources for fatty acids (FA), and in particular polyunsaturated fatty acids (PUFA), analysis of 61 species, comprising of 11 Chlorophyta, 17 Phaeophyceae (Ochrophyta) and 33 Rhodophyta, was conducted. Total fatty acid (TFA) concentrations varied considerably (between 0.6 and 7.8 in % of dry weight (DW)) between species, with on average the highest concentrations being in the Phaeophyceae, then the Chlorophyta, and with the Rhodophyta recording the lowest average concentrations. Results revealed significant differences in the fatty acid profiles of the three algal groups. Most species exhibit high proportions of PUFA in their fatty acid profile and a low ratio of n-6/n-3 PUFA. These properties highlight the potential for southern-Australian macroalgae to be used for these FA in food, animal feed and nutraceutical applications.

A novel approach for extraction of algal oil from marine macroalgae *Ulva fasciata*

Source:Renewable Energy, Volume 127

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In this present investigation, lipid extraction was performed in marine macro algae *Ulva fasciata* using soxhlet and super critical fluid extraction apparatus. The ultrasonic pretreatment was done for the algal biomass. Extraction parameters for High extraction yield of algal oil were obtained at 5% moisture content, 0.25 mm particle size, 90 °C, 180 min time, 5:1 solvent-to-solid ratio and solvent flow rate 1l/hr. After the optimization process 9.85% of oil extraction was obtained from *Ulva fasciata* algal biomass. The aromatic groups, indicating the presence of 12-Hydroxy-9-octadecenoic acid was confirmed by FT-IR analysis. Thus the algal oil obtained from *Ulva fasciata* biomass can be used for the biodiesel production. The saturated fatty acid (SFAs) was found to be 75.43% in the produced algal oil. The properties like Acid value, Iodine number, specific gravity, Saponification value, calorific value, Flash point, Fire point and kinematic viscosity was also characterized.



PROCESS OF PRODUCTION OF OIL FROM MICROALGAE

CANEPA PIETRO [IT],

The invention relates to a process for the production of lipids (oil) from microalgae, comprising the cultivation of microalgae by sequential photoautotrophic-heterotrophic growth, wherein in the heterotrophic step the microalgae are fed by administration of a sugar feed deriving from sugar production waste, for example molasses or bagasse, or else deriving from waste from the fruit candying industry, for example candying water, which has a sugar content comprised between 20 % and 60 % by weight. The invention also relates to a plant for the production of lipids (oil) from microalgae, intended for carrying out the process of the invention.

Method for Biodiesel Manufacturing using Non-catalytic Esterification Reaction from Microalgae

LEE JAE W [US]; KIM BO RA [KR]

The present invention relates to a method for manufacturing bio-diesel by extracting lipid from microalgae, which is not processed by a drying process, using alcohol without using a catalyst, and performing non-catalytic esterification reaction and trans-esterification reaction. According to the present invention, the method for manufacturing bio-diesel from microalgae by using alcohol does not apply a process for drying microalgae and thus requires low energy consumption and low process costs needed for facilities. Since a strong acid catalyst such as sulfuric acid, hydrochloric acid, or nitric acid is not used, the method for manufacturing bio-diesel generates little environmental burden such as waste water treatment. Accordingly, the method for manufacturing bio-diesel can reduce production costs of bio-diesel based on microalgae.

Joint treatment device based on integration of vapor recompression and heat exchange and applied to microalgae

SONG CHUNFENG; XIE MEILIAN (5)

The invention relates to the technical field of microalgae treatment with high energy efficiency, in particular to a joint treatment device based on integration of vapor recompression and heat exchange and applied to microalgae. The joint treatment device comprises a microalgae drying system, a grease extraction system and a solvent recovery system, wherein each of the microalgae drying system and the solvent recovery system adopts a vapor recompression step. Gas at the top end of a dryer is subjected to vapor recompression, the gas enters a second-stage heat exchanger to exchange heat with wet algae, and meanwhile, dry algae obtained after drying exchanges energy with the wet algae. By performing vapor recompression in a microalgae drying process, the energy efficiency is increased and energy exchange with the wet algae is realized, and further, energy interexchange of the wet algae and the dry algae is realized; in a solvent recovery stage, energy at the top of a fractionating column is recovered and introduced into a compressor, so that energy of gas flow at the top of the fractionating



column is recovered to heat a pre-distillation mixture and grease/solvent at the bottom of the fractionating column, energy efficiency is increased, and a preheater and a reboiler are omitted.

TWO-STAGE PROCESS FOR PRODUCING OIL FROM MICROALGAE

OYLER JAMES R [US]

A process for production of biofuels from algae can include cultivating an oil-producing algae by promoting sequential photoautotrophic and heterotrophic growth. The method can further include producing oil by heterotrophic growth of algae wherein the heterotrophic algae growth is achieved by introducing a sugar feed to the oil-producing algae. An algal oil can be extracted from the oil-producing algae, and can be converted to form biodiesel.

PROCESS FOR PRODUCING STARCH FROM MICROALGAE

PAGNANELLI FRANCESCA [IT]; TORO LUIGI [IT] (2)

The process described relates to the production of starch from microalgae through cultivation in an autotrophic stage followed by a subsequent heterotrophic stage. The autotrophic stage is used to produce the inoculum for cultivation under heterotrophic conditions. In the heterotrophic reactor starch accumulates in the algae. Cultivation under heterotrophic conditions is carried out with the addition of antimicrobials of natural origin of the phenol type on a growth medium such as for example one based on whey, in order to reduce contamination by bacteria and fungi. During this stage the starch content in the algae is monitored in order to maximise output. The microalgae used in the process are suitably selected for growth under the conditions reported. Oil and carotenoids are also extracted from the biomass in addition to starch. The process provides a method for the more efficient production of starch in comparison with conventional production and a method for controlling contaminants under heterotrophic growth conditions.

Overcompensation cultural method improving content of proteins of heterotrophic microalgae

XIE TONGHUI; ZENG YU (1)

The invention discloses a cultural method of obtaining heterotrophic chlorella cells with a high protein content. The operating method comprises the following steps of: heterotrophically culturing chlorella to an initial period of a growing logarithmic phase by using a nitrogen deficiency modified BG11+ organic culture medium; centrifugally collecting the algae cells in a sterile condition; and transferring the algae cells to a nitrogen sufficiency modified BG11+ organic culture medium to be continuously cultured to an initial period of a stable period to collect the algae with the high protein content. On the premise of guaranteeing the heterotrophic high biomass of the chlorella, the method improves the protein content in the heterotrophic chlorella living bodies, so that the output of the proteins is improved; and moreover, the produced single-cell proteins are reasonable in biochemical composition, and amino acid components meet the nutritional demand on foods and feeds. The method disclosed by the invention provides a novel technical route for improving the output of the single-cell proteins. The process has the advantages of being simple in process, simple to operate, short in production period, high in product quality and the like, and is easy for scaled production.



Method for preparing aquaculture water purifier

HUANG KAIYAO; CHEN SONGLIN (1),

The invention provides a method for preparing an aquaculture water purifier. The method comprises the following steps: (1) inoculating chlorella in a liquid culture medium, culturing for 5-7 days under the culturing condition that the temperature is 25-28 DEGC, the light intensity is 5000+/-500Lx and the pH value is 6-7; (2) centrifugally collecting chlorella sludge with water content of 40-60 percent; (3) adding a protecting agent, and adding the protecting agent into the chlorella sludge; and (4) drying and grinding, drying the chlorella sludge prepared in the step (3) to enable the water content to be 2-6 percent. According to the method, chlorella is used as a main body, and three main process flows including large-scale production, protecting agent addition and drying are adopted, so that the survival rate of chlorella cells can be increased. The water purifier has a good degrading effect on general nitrogen, general phosphorous and chemical oxygen demand of the aquaculture water body.

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