

UNIDAD DE VIGILANCIA TECNOLÓGICA E INTELIGENCIA COMPETITIVA

Microalgas

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Contenido

PUBLICACIONES	8
Effects of Nitrogen Supplementation Status on CO ₂ Biofixation and Biofuel Production of the Promising Microalga <i>Chlorella</i> sp. ABC-001.	8
Sustainable environmental management and related biofuel technologies.....	8
Pilot scale wastewater treatment, CO ₂ sequestration and lipid production using microalga, <i>Neochloris aquatica</i> RDS02.	9
Application of Static Magnetic Fields on the Mixotrophic Culture of <i>Chlorella minutissima</i> for Carbohydrate Production.....	9
Energy-efficient pretreatments for the enhanced conversion of microalgal biomass to biofuels.	10
Optimizing real swine wastewater treatment efficiency and carbohydrate productivity of newly microalga <i>Chlamydomonas</i> sp. QWY37 used for cell-displayed bioethanol production.	10
Involvement of green technology in microalgal biodiesel production.	11
Hybrid Ni@ZnO@ZnS-Microalgae for Circular Economy: A Smart Route to the Efficient Integration of Solar Photocatalytic Water Decontamination and Bioethanol Production.	11
Co-production of biodiesel and bioethanol using psychrophilic microalga <i>Chlamydomonas</i> sp. KNM0029C isolated from Arctic sea ice.....	12
Domestic wastewater treatment by constructed wetland and microalgal treatment system for the production of value-added products.....	13
Biocomponent-based microalgal transformations into biofuels during the pretreatment and fermentation process.....	13
Simultaneous saccharification and fermentation of <i>Spirulina</i> sp. and corn starch for the production of bioethanol and obtaining biopeptides with high antioxidant activity.	14
Deoiled algal biomass derived renewable sugars for bioethanol and biopolymer production in biorefinery framework.	14
Algae as green energy reserve: Technological outlook on biofuel production.....	15
Mixotrophic cultivation of <i>Spirulina platensis</i> in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity.	15
Utilization of <i>Scenedesmus obliquus</i> Protein as a Replacement of the Commercially Available Fish Meal Under an Algal Refinery Approach.....	16
Process optimization for the production of high-concentration ethanol with <i>Scenedesmus raciborskii</i> biomass.	17
<i>Scenedesmus obliquus</i> in poultry wastewater bioremediation.....	17



Effect of Chemical Pre-treatments on Bioethanol Production from <i>Chlorella minutissima</i> ...	18
Overexpression of a glycogenin, CmGLG2, enhances floridean starch accumulation in the red alga <i>Cyanidioschyzon merolae</i>	18
Assessment of biomass potentials of microalgal communities in open pond raceways using mass cultivation.....	19
Bio-electrochemical COD removal for energy-efficient, maximum and robust nitrogen recovery from urine through membrane aerated nitrification.....	19
Physiological response and oxidative transformation of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) by a <i>Chlorella</i> isolate.	20
Isoprostanoïd Profiling of Marine Microalgae.	21
Astaxanthin biosynthesis promotion with pH shock in the green microalga, <i>Haematococcus lacustris</i>	21
A proof of concept study for wastewater reuse using bioelectrochemical processes combined with complementary post-treatment technologies.....	22
Bioenergy from biofuel residues and waste.....	22
Mixotrophic cultivation of <i>Chlorella</i> for biomass production by using pH-stat culture medium: Glucose-Acetate-Phosphorus (GAP).	23
Algae-Bacteria Consortia as a Strategy to Enhance H ₂ Production.	23
Purple phototrophic bacteria for resource recovery: Challenges and opportunities.	24
On the pyrolysis of different microalgae species in a conical spouted bed reactor: Bio-fuel yields and characterization.....	24
Sulfonamides-induced oxidative stress in freshwater microalga <i>Chlorella vulgaris</i> : Evaluation of growth, photosynthesis, antioxidants, ultrastructure, and nucleic acids.	25
Adsorption of Pb ²⁺ onto freeze-dried microalgae and environmental risk assessment.....	25
Autotrophic granulation of hydrogen consumer denitrifiers and microalgae for nitrate removal from drinking water resources at different hydraulic retention times.....	26
Hydrothermal liquefaction of <i>Scenedesmus obliquus</i> using a novel catalyst derived from clam shells: Solid residue as catalyst for hydrogen production.	27
Energy conservation in photosynthetic microorganisms.	27
Influence of <i>Microcystis</i> sp. and freshwater algae on pH: Changes in their growth associated with sediment.....	28
Valorization of waste eggshell-derived bioflocculant for harvesting <i>T. obliquus</i> : Process optimization, kinetic studies and recyclability of the spent medium for circular bioeconomy.	28
Overproduction of the Flv3B flavodiiron, enhances the photobiological hydrogen production by the nitrogen-fixing cyanobacterium <i>Nostoc</i> PCC 7120.....	29
Analysis of the energy barrier between <i>Chlorella vulgaris</i> cells and their interfacial interactions with cationic starch under different pH and ionic strength.....	29
Understanding the by-product formation potential during phenol oxidation from in-situ electro-generated radicals by microalgae harvesting.	30
Evaluation of hydrogen yield potential from <i>Chlorella</i> by photo-fermentation under diverse substrate concentration and enzyme loading.....	30



Po uptake in microalgae at different seawater pH: An experimental study simulating ocean acidification.	31
Toxicity of microwave-synthesized silver-reduced graphene oxide nanocomposites to the microalga <i>Chlorella vulgaris</i> : Comparison with the hydrothermal method synthesized counterparts.	31
Microalgae carbon fixation integrated with organic matters recycling from soybean wastewater: Effect of pH on the performance of hybrid system.....	32
Simultaneous biohydrogen production from dark fermentation of duckweed and waste utilization for microalgal lipid production.	32
Co-Hydrothermal gasification of <i>Chlorella vulgaris</i> and hydrochar: The effects of waste-to-solid biofuel production and blending concentration on biogas generation.....	33
Pretreatment of microalgal biomass for efficient biohydrogen production - Recent insights and future perspectives.....	33
Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis.	34
Temperate-zone cultivation of <i>Oedogonium</i> in municipal wastewater effluent to produce cellulose and oxygen.	34
Conversion and Stability of New Metabolites of Paralytic Shellfish Toxins under Different Temperature and pH Conditions.	35
Photoautotrophic cultures of <i>Chlamydomonas reinhardtii</i> : sulfur deficiency, anoxia, and hydrogen production.....	35
The use of response surface methodology for improving fatty acid methyl ester profile of <i>Scenedesmus vacuolatus</i>	36
In Situ Microscopy for Real-time Determination of Single-cell Morphology in Bioprocesses.	37
Metabolic responses of the green microalga <i>Dunaliella salina</i> to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment.....	37
Microalgae harvesting from wastewater by pH modulation and flotation: Assessing and optimizing operational parameters.....	38
Amyloid protein produced by <i>B. cereus</i> CR4 possesses bioflocculant activity and has potential application in microalgae harvest.....	39
Development and Characterization of Astaxanthin-Containing Whey Protein-Based Nanoparticles.	39
Archaea inhibition: Strategies for the enhancement of volatile fatty acids production from microalgae.	40
Tear Down the Fluorescent Curtain: A New Fluorescence Suppression Method for Raman Microspectroscopic Analyses.	40
Hybrid liquid biphasic system for cell disruption and simultaneous lipid extraction from microalgae <i>Chlorella sorokiniana</i> CY-1 for biofuel production.	41
Sensory properties of aqueous dispersions of protein-rich extracts from <i>Chlorella protothecoides</i> at neutral and acidic pH.....	41
Aged microplastics polyvinyl chloride interact with copper and cause oxidative stress towards microalgae <i>Chlorella vulgaris</i>	42



Combinational system for biodegradation of olive oil mill wastewater phenolics and high yield of bio-hydrogen production.....	42
Effects of <i>Chlorella vulgaris</i> polysaccharides accumulation on growth characteristics of <i>Trachemys scripta elegans</i>	43
Adsorption thermodynamic characteristics of <i>Chlorella vulgaris</i> with organic polymer adsorbent cationic starch: Effect of temperature on adsorption capacity and rate.	44
Effects of carbon concentration, pH, and bubbling depth on carbon dioxide absorption ratio in microalgae medium.....	44
Extraction, structural and functional properties of <i>Haematococcus pluvialis</i> protein after pigment removal.	45
Cultivation of <i>scenedesmus</i> sp. using optimized minimal nutrients and flocculants - a potential platform for mass cultivation.	45
Regulation of biohydrogen production by protonophores in novel green microalgae <i>Parachlorella kessleri</i>	46
Ecotoxicological and biochemical effects of environmental concentrations of the plastic-bond pollutant dibutyl phthalate on <i>Scenedesmus</i> sp.....	46
Comparison of algal harvest and hydrogen peroxide treatment in mitigating cyanobacterial blooms via an in situ mesocosm experiment.	47
Nannochloropsis Extract-Mediated Synthesis of Biogenic Silver Nanoparticles, Characterization and In Vitro Assessment of Antimicrobial, Antioxidant and Cytotoxic Activities.	48
Immobilization of mercury using high-phosphate culture-modified microalgae.	48
pH effects on the lipid and fatty acids accumulation in <i>Chlamydomonas reinhardtii</i>	49
Optimization, isolation, characterization and hepatoprotective effect of a novel pigment-protein complex (phycocyanin) producing microalga: <i>Phormidium versicolor</i> NCC-466 using response surface methodology.....	49
Graphene-based materials do not impair physiology, gene expression and growth dynamics of the aeroterrestrial microalga <i>Trebouxia gelatinosa</i>	50
Impact of Culture Conditions on Neutral Lipid Production by Oleaginous Yeast.	51
Optimization of pH induced flocculation of marine and freshwater microalgae via central composite design.....	51
Eco-friendly rapid removal of triclosan from seawater using biomass of a microalgal species: Kinetic and equilibrium studies.	52
Solubility and aggregation behavior of protein fractions from the heterotrophically cultivated microalga <i>Chlorella protothecoides</i>	52
A water-soluble [60]fullerene-derivative stimulates chlorophyll accumulation and has no toxic effect on <i>Chlamydomonas reinhardtii</i>	53
The effect of pH on the acute toxicity of phenanthrene in a marine microalgae <i>Chlorella salina</i>	53
Growth and photosynthetic activity of <i>Chlamydomonas reinhardtii</i> entrapped in lens-shaped silica hydrogels.	54
EVENTOS	55



Árbol de categorías	55
Español	55
Inglés.....	56



PUBLICACIONES

Effects of Nitrogen Supplementation Status on CO₂ Biofixation and Biofuel Production of the Promising Microalga *Chlorella* sp. ABC-001.

J Microbiol Biotechnol. 2020 Aug 28;30(8):1235-1243

Authors: Cho JM, Oh YK, Park WK, Chang YK

Abstract

The use of microalgal biomass as feedstock for biofuels has been discussed for decades as it provides a sustainable approach to producing fuels for the future. Nonetheless, its feasibility has not been established yet and various aspects of biomass applications such as CO₂ biofixation should also be explored. Therefore, in this study, the CO₂ biofixation and lipid/carbohydrate production potential of *Chlorella* sp. ABC-001 were examined under various nitrogen concentrations. The highest biomass productivity and CO₂ biofixation rate of 0.422 g/l/d and 0.683 g/l/d, respectively, were achieved under a nitrogen-rich condition (15 mM nitrate). Carbohydrate content was generally proportional to initial nitrate concentration and showed the highest value of 41.5% with 15 mM. However, lipid content showed an inverse relationship with nitrogen supplementation and showed the highest value of 47.4% with 2.5 mM. In consideration as feedstock for biofuels (bioethanol, biodiesel, and biogas), the sum of carbohydrate and lipid contents were examined and the highest value of 79.6% was achieved under low nitrogen condition (2.5 mM). For lipid-based biofuel production, low nitrogen supplementation should be pursued. However, considering the lower feasibility of biodiesel, pursuing CO₂ biofixation and the production of carbohydrate-based fuels under nitrogenrich condition might be more rational. Thus, nitrogen status as a cultivation strategy must be optimized according to the objective, and this was confirmed with the promising alga *Chlorella* sp. ABC-001.

Sustainable environmental management and related biofuel technologies.

J Environ Manage. 2020 Jul 30;273:111096

Authors: Sharma S, Kundu A, Basu S, Shetti NP, Aminabhavi TM

Abstract

Environmental sustainability criteria and rising energy demands, exhaustion of conventional resources of energy followed by environmental degradation due to abrupt climate changes have shifted the attention of scientists to seek renewable sources of green and clean energy for



sustainable development. Bioenergy is an excellent alternative since it can be applied for several energy-requirements after utilizing suitable conversion methodology. This review elucidates all aspects of biofuels (bioethanol, biodiesel, and butanol) and their sustainability criteria. The principal focus is on the latest developments in biofuel production chiefly stressing on the role of nanotechnology. A plethora of investigations regarding the emerging techniques for process improvement like integration methods, less energy-intensive distillation techniques, and bioengineering of microorganisms are discussed. This can assist in making biofuel-production in a real-world market more economically and environmentally viable.

Pilot scale wastewater treatment, CO₂ sequestration and lipid production using microalga, *Neochloris aquatica* RDS02.

Int J Phytoremediation. 2020 Jul 02;;1-18

Authors: Tamil Selvan S, Velramar B, Ramamurthy D, Balasundaram S, Sivamani K

Abstract

In present investigation carried out large-scale treatment of tannery effluent by the cultivation of microalgae, *Neochloris aquatica* RDS02. The tannery effluent treatment revealed that significant reduction heavy metals were chromium-3.59, lead-2.85, nickel-1.9, cadmium-10.68, zinc-4.49, copper-0.95 and cobalt-1.86 mg/L on 15th day of treatment using *N. aquatica* RDS02. The microalgal biosorption capacity q_{max} rate was Cr-88.66, Pb-75.87, Ni-87.61, Cd-60.44, Co-52.86, Zn-84.90 and Cu-54.39, and isotherm model emphasized that the higher R² value 0.99 by Langmuir and Freundlich kinetics model. The microalga utilized highest CO₂ (90%) analyzed by CO₂ biofixation and utilization kinetics, biomass (3.9 mg/mL), lipid (210 mg mL⁻¹), carbohydrate (102.75 mg mL⁻¹), biodiesel (4.9 mL g⁻¹) and bioethanol (4.1 mL g⁻¹). The microalgal-lipid content was analyzed through Nile red staining. Gas chromatography mass spectrometric (GCMS) analysis confirmed that the presence of a biodiesel and major fatty acid methyl ester (FAME) profiling viz., tridecanoic acid methyl ester, pentadecanoic acid methyl ester, octadecanoic acid methyl ester, myristic acid methyl ester, palmitic acid methyl ester and oleic acid methyl ester. Fourier transform infrared (FTIR) analysis confirmed that the presence of a functional groups viz., phenols, alcohols, alkynes, carboxylic acids, ketones, carbonyl and ester groups. The bioethanol production was confirmed by high-performance liquid chromatography (HPLC) analyze.

Application of Static Magnetic Fields on the Mixotrophic Culture of *Chlorella minutissima* for Carbohydrate Production.

Appl Biochem Biotechnol. 2020 Jun 29;;

Authors: Menestrino BDC, Pintos THC, Sala L, Costa JAV, Santos LO



Abstract

Magnetic field (MF) can interact with the metabolism of microalgae and has an effect (positive or negative) on the synthesis of molecules. In addition to MF, the use of pentose as a carbon source for cultivating microalgae is an alternative to increase carbohydrate yield. This study aimed at evaluating the MF application on the mixotrophic culture of *Chlorella minutissima* in order to produce carbohydrates. MF of 30 mT was generated by ferrite magnets and applied diurnally for 12 days. The addition of 5% pentose, MF application of 30 mT, and nitrogen concentration reduced (1.25 mM of KNO₃) was the best conditions to obtain higher carbohydrate concentrations. MF application of 30 mT increased biomass and carbohydrate contents in 30% and 163.1%, respectively, when compared with the assay without MF application. The carbohydrate produced can be used for bioethanol production.

Energy-efficient pretreatments for the enhanced conversion of microalgal biomass to biofuels.

Bioresour Technol. 2020 Aug;309:123333

Authors: Ha GS, El-Dalatony MM, Kurade MB, Salama ES, Basak B, Kang D, Roh HS, Lim H, Jeon BH

Abstract

The physiological properties, including biochemical composition and cell wall thickness, of microalgal species have a remarkable effect on the pretreatment of biomass and its further conversion to biofuels. In the present study, multiple biofuels (bioethanol, higher alcohols (C₃-C₅), and biodiesel) were produced using energy-efficient microwave pretreatment, successive carbohydrate/protein fermentation, and lipid transesterification from three microalgal strains (*Pseudochlorella* sp., *Chlamydomonas mexicana*, and *Chlamydomonas pitschmannii*). The microwave pretreatment method required the lowest specific energy (5 MJ/kg) compared to ultrasound pretreatment. The proposed integrated approach achieved high conversion efficiency (46%) and maximum biomass utilization (93%) of *C. mexicana* with improved yields of bioethanol (0.46 g-ethanol/g-carbohydrates), higher alcohols (0.44 g-higher alcohols/g-proteins), and biodiesel (0.74 g-biodiesel/g-lipids). This study suggests that the application of an appropriate pretreatment method for microalgal strains having different physiological properties is essential for improving the extraction efficiency and conversion of biomass to biofuels with less waste production.

Optimizing real swine wastewater treatment efficiency and carbohydrate productivity of newly microalga *Chlamydomonas* sp. QWY37 used for cell-displayed bioethanol production.

Bioresour Technol. 2020 Feb 22;305:123072

Authors: Qu W, Loke Show P, Hasunuma T, Ho SH



Abstract

This work aimed to study an newly isolated microalgal strain, *Chlamydomonas* sp. QWY37, that can achieve a maximum carbohydrate production of 944 mg/L·d, along with high pollutant removal efficiencies (chemical oxygen demand: 81%, total nitrogen: 96%, total phosphate: nearly 100%) by optimizing culture conditions and using an appropriate operation strategy. Through a cell-displayed technology that utilizes combined engineered system, a maximum microalgal bioethanol yield of 61 g/L was achieved. This is the first report demonstrating the highest microalgal carbohydrate productivity using swine wastewater without any pretreatments associated with direct high-density bioethanol production from the subsequent microalgal biomass. This work may represent a breakthrough in achieving feasible microalgal bioethanol conversion from real swine wastewater.

Involvement of green technology in microalgal biodiesel production.

Rev Environ Health. 2020 Jun 25;35(2):173-188

Authors: Verma S, Kuila A

Abstract

According to the report of the renewable energy policy network for the 21st century published in 2014, biodiesel and bioethanol are the most used biofuels and are responsible for transportation worldwide. Biodiesel specially has shown an increase in production globally by 15 times by volume from 2002 to 2012. Promising feedstock of biodiesel are cyanobacteria and microalgae as they possess a shorter cultivation time (4 fold lesser) and high oil content (10 fold higher) than corn, jatropha and soybean (conventional oil-producing territorial plants). Various valuable natural chemicals are also produced from these organisms including food supplements such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), pigments, and vitamins. Additionally, cellular components of microalgae and cyanobacteria are connected with therapeutic characteristics such as anti-inflammatory, antioxidant, antiviral and immune stimulating. Commercialization of algal biodiesel (or other products) can be achieved by isolating and identifying the high-yielding strains that possess a faster growth rate. Indigenous strains can be genetically engineered into high-yielding transgenic strains. The present article discusses about the use of nanotechnology and genetic engineering approach for improved lipid accumulation in microalgae for biodiesel production.

Hybrid Ni@ZnO@ZnS-Microalgae for Circular Economy: A Smart Route to the Efficient Integration of Solar Photocatalytic Water Decontamination and Bioethanol Production.

Adv Sci (Weinh). 2020 Feb;7(3):1902447

Authors: Serrà A, Artal R, García-Amorós J, Sepúlveda B, Gómez E, Nogués J, Philippe L



Abstract

Water remediation and development of carbon-neutral fuels are a priority for the evermore industrialized society. The answer to these challenges should be simple, sustainable, and inexpensive. Thus, biomimetic-inspired circular and holistic processes combining water remediation and biofuel production can be an appealing concept to deal with these global issues. A simple circular approach using helical *Spirulina platensis* microalgae as biotemplates to synthesize Ni@ZnO@ZnS photocatalysts for efficient solar water decontamination and bioethanol production during the recycling process is presented. Under solar irradiation, the Ni@ZnO@ZnS-Spirulina photocatalyst exhibits enhanced activity (mineralization efficiency >99%) with minimal photocorrosion and excellent reusability. At the end of its effective lifetime for water remediation, the microalgae skeleton (mainly glycogen and glucose) of the photocatalyst is recycled to directly produce bioethanol by simultaneous saccharification and fermentation process. An outstanding ethanol yield of 0.4 L kg⁻¹, which is similar to the highest yield obtained from oxygenic photosynthetic microorganisms, is obtained. Thus, the entire process allows effective solar photocatalytic water remediation and bioethanol production at room temperature using simple and easily scalable procedures that simultaneously fixes carbon dioxide, thereby constituting a zero-carbon-emission circular process.

Co-production of biodiesel and bioethanol using psychrophilic microalga *Chlamydomonas* sp. KNM0029C isolated from Arctic sea ice.

Biotechnol Biofuels. 2020;13:20

Authors: Kim EJ, Kim S, Choi HG, Han SJ

Abstract

Background: Biofuels, generated using microalgae as sustainable energy, have received a lot of attention. Microalgae can be cultivated at low cost with CO₂ and solar energy without competition from edible crops. Psychrophilic microalgae can be a suitable feedstock to produce biofuels without the environmental constraints of low temperatures, because they can grow below 10 °C. However, there is a lack of efficient strategies using psychrophilic microalgae to produce biodiesel and bioethanol. Therefore, the current study aimed to optimize the production of biodiesel and bioethanol from Arctic *Chlamydomonas* sp. KNM0029C at low temperatures. **Results:** After incubation in a 20-L photobioreactor, fatty acid methyl ester (FAME) was extracted using modified FAME extraction methods, producing a maximum yield of 0.16-g FAME/g KNM0029C. Residual biomass was pretreated for bioethanol production, and the yields from different methods were compared. The highest bioethanol yield (0.22-g/g residual biomass) was obtained by pretreatment with enzyme (amylglucosidase) after sonication. Approximately 300-mg biofuel was obtained, including 156-mg FAME biodiesel and 144-mg bioethanol per g dried cells, representing the highest recorded yield from psychrophilic microalgae.

Conclusions: This is the first to attempt at utilizing biomass from psychrophilic Arctic microalga *Chlamydomonas* sp. KNM0029C for the co-production of bioethanol and biodiesel, and it yielded the highest values among reported studies using psychrophilic organisms. These results can be used as a source for the efficient biofuel production using polar microalgae.



Domestic wastewater treatment by constructed wetland and microalgal treatment system for the production of value-added products.

Authors: Chavan R, Mutnuri S

Abstract

The main aim of this study is to treat domestic wastewater in a hybrid Vertical Flow Constructed Wetland (VFCW-4.2 m²) and Microalgal Treatment System (MTS-1 m²). The objective is not only to treat Domestic wastewater (DW) but also to produce value-added products from microalgal biomass. The domestic wastewater was initially treated by VFCW and the VFCW effluent was further phycoremediated by MTS. *Canna indica* was used for wetland vegetation and resident microalgal consortium from VFCW effluent was used in MTS. The VFCW and MTS was operated at 1 m³/day (HRT-0.25 m³/m²-day, OLR-400 g/m²-day) and 0.03 m³/day (HRT-0.03 m³/m²-day, OLR-400 g/m²-day), respectively. The integrated system was observed to remove 68.9% COD, 77.4% NH₄-N, 75.8% TKN and 63.6% PO₄-P. The harvested Naive Biomass (NB) was observed to contain 16.7% of lipids (W/W). The Residual Biomass after Lipid Extraction (RBLE) was used as a substrate for ethanol production. The observed yield of ethanol using RBLE as a substrate was 33.4%. NB, RBLE, and Residual Biomass after Lipid and Sugar Extraction (RBLSE) indicated net biomethane yield (mL/g VS) of 211.8, 134.6 and 107.7, respectively. The present study demonstrated an initial attempt of demonstrating a hybrid wastewater treatment system for the production of value-added products in terms of biofuel.

Biocomponent-based microalgal transformations into biofuels during the pretreatment and fermentation process.

Bioresour Technol. 2020 Apr;302:122809

Authors: Ha GS, El-Dalatony MM, Kim DH, Salama ES, Kurade MB, Roh HS, El-Fatah Abomohra A, Jeon BH

Abstract

Microalgal cell wall integrity and composition have a significant impact on the fermentation process and biofuel recovery. In this study, various biofuels (bioethanol, higher alcohols (C₃-C₅), and biodiesel) were produced by the fermentation of carbohydrates and proteins, and transesterification of lipids from three different microalgal strains (*Pseudochlorella* sp., *Chlamydomonas mexicana*, and *Chlamydomonas pitschmannii*), each possessing different proportions of bioconstituents (carbohydrates, proteins, and lipids). Changes in the cell wall structure and thickness were observed before and after fermentation using transmission electron microscopy. *Pseudochlorella* sp. showed the highest yields of bioethanol (0.45 g-ethanol/g-carbohydrates), higher alcohols (0.44 g-higher alcohols/g-proteins), and biodiesel (0.55 g-biodiesel/g-lipids), which consequently revealed a maximum energy recovery (42%) from whole constituents. This study suggests that different physiological properties, including



cell wall thickness and the proportion of bioconstituents in microalgae, could have a significant impact on the pretreatment and fermentation efficiencies for biofuels production.

Simultaneous saccharification and fermentation of *Spirulina* sp. and corn starch for the production of bioethanol and obtaining biopeptides with high antioxidant activity.

Bioresour Technol. 2020 Apr;301:122698

Authors: Luiza Astolfi A, Rempel A, Cavanhi VAF, Alves M, Deamici KM, Colla LM, Costa JAV

Abstract

The aim was to produce bioethanol by the simultaneous saccharification and fermentation (SSF) of *Spirulina* sp. LEB 18 biomass and corn starch, increasing the process scale and obtaining biopeptides from bioethanol residue. Different temperatures of SSF and biomass/starch concentrations were tested, and the best conditions were chosen to scale-up the bioethanol production. The biopeptides were obtained enzymatically with a protease. The antioxidant capacity, molecular structure, thermal stability and mass loss of the biopeptides were evaluated. A total of 73 g L⁻¹ bioethanol was obtained during scale-up, and the residue presented a high protein content with a degree of hydrolysis of 86%. The biopeptides showed 32% ABTS radical inhibition with high thermal stability. This study showed the possibility of the biorefinery concept being able to produce bioethanol by *Spirulina*, and the biopeptides from the bioethanol residue presented high antioxidant capacity and can be used in many areas of the food industry.

Deoiled algal biomass derived renewable sugars for bioethanol and biopolymer production in biorefinery framework.

Bioresour Technol. 2020 Jan;296:122315

Authors: Naresh Kumar A, Chatterjee S, Hemalatha M, Althuri A, Min B, Kim SH, Venkata Mohan S

Abstract

The present study is designed to evaluate the potential of deoiled algal biomass (DAB) residue as an alternative resource for the production of bioethanol and biopolymers in a biorefinery approach. Hybrid pretreatment method resulted in higher sugar solubilization (0.590 g/g DAB) than the corresponding individual physicochemical (0.481 g/g DAB) and enzymatic methods (0.484 g/g DAB). Subsequent utilization of sugars from hybrid pretreatment for bioethanol using *Saccharomyces cerevisiae* resulted in maximum bioethanol production at pH 5.5 (0.145 ± 0.008 g/g DAB) followed by pH 5.0 (0.122 ± 0.004 g/g DAB) and pH 6.0 (0.102 ± 0.002 g/g DAB). The experiments for biopolymer (PHB: polyhydroxybutyrate) production resulted in 0.43 ± 0.20 g PHB/g DCW. Extracted polymer on NMR and FT-IR analysis showed the presence of PHB. Exploration of DAB as an alternative renewable resource for



multiple biobased products supports sustainability and also enables entirety use of DAB by addressing the DAB-residue allied disposal issues.

Algae as green energy reserve: Technological outlook on biofuel production.

Chemosphere. 2020 Mar;242:125079

Authors: Anto S, Mukherjee SS, Muthappa R, Mathimani T, Deviram G, Kumar SS, Verma TN, Pugazhendhi A

Abstract

Depletion of fossil fuel sources and their emissions have triggered a vigorous research in finding alternative and renewable energy sources. In this regard, algae are being exploited as a third generation feedstock for the production of biofuels such as bioethanol, biodiesel, biogas, and biohydrogen. However, algal based biofuel does not reach successful peak due to the higher cost issues in cultivation, harvesting and extraction steps. Therefore, this review presents an extensive detail of deriving biofuels from algal biomass starting from various algae cultivation systems like raceway pond and photobioreactors and its bottlenecks. Evolution of biofuel feedstocks from edible oils to algae have been addressed in the initial section of the manuscript to provide insights on the different generation of biofuel. Different configuration of photobioreactor systems used to reduce contamination risk and improve biomass productivity were extensively discussed. Photobioreactor performance greatly relies on the conditions under which it is operated. Hence, the importance of such conditions alike temperature, light intensity, inoculum size, CO₂, nutrient concentration, and mixing in bioreactor performance have been described. As the lipid is the main component in biodiesel production, several pretreatment methods such as physical, chemical and biological for disrupting cell membrane to extract lipid were comprehensively reviewed and presented. This review article had put forth the recent advancement in the pretreatment methods like hydrothermal processing of algal biomasses using acid or alkali. Eventually, challenges and future dimensions in algal cultivation and pretreatment process were discussed in detail for making an economically viable algal biofuel.

Mixotrophic cultivation of *Spirulina platensis* in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity.

PLoS One. 2019;14(10):e0224294

Authors: Pereira MIB, Chagas BME, Sassi R, Medeiros GF, Aguiar EM, Borba LHF, Silva EPE, Neto JCA, Rangel AHN

Abstract

Mixotrophic cultivation of microalgae provides a very promising alternative for producing



carbohydrate-rich biomass to convert into bioethanol and value-added biocompounds, such as vitamins, pigments, proteins, lipids and antioxidant compounds. *Spirulina platensis* may present high yields of biomass and carbohydrates when it is grown under mixotrophic conditions using cheese whey. However, there are no previous studies evaluating the influence of this culture system on the profile of fatty acids or antioxidant compounds of this species, which are extremely important for food and pharmaceutical applications and would add value to the cultivation process. *S. platensis* presented higher specific growth rates, biomass productivity and carbohydrate content under mixotrophic conditions; however, the antioxidant capacity and the protein and lipid content were lower than that of the autotrophic culture. The maximum biomass yield was 2.98 ± 0.07 g/L in growth medium with 5.0% whey. The phenolic compound concentration was the same for the biomass obtained under autotrophic and mixotrophic conditions with 2.5% and 5.0% whey. The phenolic compound concentrations showed no significant differences except for that in the growth medium with 10.0% whey, which presented an average value of 22.37 ± 0.14 mg gallic acid/g. Mixotrophic cultivation of *S. platensis* using whey can be considered a viable alternative to reduce the costs of producing *S. platensis* biomass and carbohydrates, shorten cultivation time and produce carbohydrates, as it does not require adding expensive chemical nutrients to the growth medium and also takes advantage of cheese whey, an adverse dairy industry byproduct.

Utilization of *Scenedesmus obliquus* Protein as a Replacement of the Commercially Available Fish Meal Under an Algal Refinery Approach.

Front Microbiol. 2019;10:2114

Authors: Patnaik R, Singh NK, Bagchi SK, Rao PS, Mallick N

Abstract

The approach of algal refinery as a method to reduce the cost of algal biodiesel by co-production of various value-added chemicals is the most up-coming strategy suggested for the economic viability of microalgal biodiesel. This concept being relatively new and novel, abundant literature on the subject is not available although fragmented data on some feedstocks are present. The main objective of this research paper is to propose an algal refinery design through utilization of *Scenedesmus obliquus* biomass for production of various industrially important products. For this purpose, first a protocol was standardized for maximum extraction of protein from *S. obliquus* biomass. Then, different experiments were conducted for 90 days each to find out the optimum concentration of microalgal protein that can be substituted in the diets of freshwater fishes for their maximum growth. During these experiments eight different growth parameters and seven water quality parameters were tested. Results showed that the standard + whole microalgal biomass + extracted microalgal protein diet (25:25:50) was the best diet for maximum growth of the freshwater fishes. After conducting these experiments, a detailed sequential extraction process for maximum valorization of the *S. obliquus* biomass or in other words an algal refinery was designed. The detailed sequential process developed, yielded 0.06 g of β -carotene, 10 g of protein, 38 g (43 mL) of biodiesel, 2 g of omega-3 fatty acid, 3 g (2.4 mL) of glycerol and 18 g (23 mL) of bioethanol from 1 Kg wet (≈ 100 g dry) *S. obliquus* biomass thus converting 70% of the test microalgal biomass into biodiesel and other value-added products by using an algal refinery approach.



Process optimization for the production of high-concentration ethanol with *Scenedesmus raciborskii* biomass.

Bioresour Technol. 2019 Dec;294:122219

Authors: Alam MA, Yuan T, Xiong W, Zhang B, Lv Y, Xu J

Abstract

Scenedesmus raciborskii WZKMT was subjected to fed-batch enzymatic hydrolysis and fermentation to facilitate the saccharification of high-solid-loading substrate for high-concentration ethanol. In this work, process factors affecting enzymatic hydrolysis, including enzyme loading, temperature, pH, and solid loading, were optimized. Results showed that 58.03 g L⁻¹ glucose, 12.57 g L⁻¹ xylose, and 1.45 g L⁻¹ cellobiose were obtained after the enzymatic hydrolysis of 330 g L⁻¹ substrates under the optimal conditions of 30 FPU g⁻¹ enzyme loading, 50 °C, and pH 5.5. Meanwhile, 89.60% yield and 30.43 g L⁻¹ content of ethanol were obtained after the fermentation of 330 g L⁻¹ hydrolysate. The maximum ethanol concentration of 79.38 g L⁻¹ could be achieved through repeated fed-batch process, indicating that *S. raciborskii* WZKMT is a promising feedstock for ethanol production.

Scenedesmus obliquus in poultry wastewater bioremediation.

Environ Technol. 2019 Dec;40(28):3735-3744

Authors: Oliveira AC, Barata A, Batista AP, Gouveia L

Abstract

Wastewater biological treatment with microalgae can be an effective technology, removing nutrients and other contaminants while reducing chemical oxygen demand. This can be particularly interesting for the meat producing industry which produces large volumes of wastewater from the slaughtering of animals and cleaning of their facilities. The main purpose of this research was the treatment of poultry wastewater using *Scenedesmus obliquus* in an economical and environmentally sustainable way. Two wastewaters were collected from a Portuguese poultry slaughterhouse (poultry raw - PR and poultry flocculated - PF) and the bioremediation was evaluated. The performance of microalga biomass growth and biochemical composition were assessed for two illumination sources (fluorescent vs LEDs). *S. obliquus* achieved positive results when grown in highly contaminated agro-industrial wastewater from the poultry industry, independently of the light source. The wastewater bioremediation revealed results higher than 97% for both ammonium and phosphate removal efficiency, for a cultivation time of 13 days. The saponifiable matter obtained from the biomass of the microalga cultures was, on average, 11% and 27% (m/malga) with PR and PF wastewater, respectively. In opposition, higher sugar content was obtained from microalgae biomass grown in PR wastewater (average 34% m/malga) in comparison to PF wastewater (average 23% m/malga), independently of the illumination source. Therefore, biomass obtained with PR wastewater will



be more appropriate as a raw material for bioethanol/biohydrogen production (higher sugar content) while biomass produced in PF wastewater will have a similar potential as feedstock for both biodiesel and bioethanol/biohydrogen production (similar lipid and sugar content).

Effect of Chemical Pre-treatments on Bioethanol Production from *Chlorella minutissima*.

Acta Chim Slov. 2018 Mar;65(1):160-165

Authors: Şerbetçioğlu Sert B, İnan B, Özçimen D

Abstract

In recent years, algal bioethanol production comes into prominence as a trend towards sustainable development. Due to being sustainable energy source and environmental friendly, bioethanol production from algae is becoming increasingly popular all over the world. However, yield of bioethanol production from algae is lower than first generation feedstock's currently, and needs to be improved. In order to increase bioethanol yield, pre-treatments should be performed as cell disruption process on algal biomass. For this reason, researchers investigate the most appropriate pre-treatment method and its parameters for high yield bioethanol production from algae. In this study, cultivated *Chlorella minutissima* was utilized for bioethanol production. Effects of pre-treatment method (dilute acid and alkaline), chemical concentration, pre-treatment temperature and pre-treatment time on bioethanol yield were investigated. It was found that, the highest bioethanol yield was obtained as 18.52% with acid pre-treatment at pre-treatment temperature of 100 °C and pre-treatment time of 60 minutes.

Overexpression of a glycogenin, CmGLG2, enhances floridean starch accumulation in the red alga *Cyanidioschyzon merolae*.

Plant Signal Behav. 2019;14(6):1596718

Authors: Pancha I, Tanaka K, Imamura S

Abstract

Microalgae accumulate energy-reserved molecules, such as triacylglycerol and carbohydrates, which are suitable feedstocks for renewable energies such as biodiesel and bioethanol. However, the molecular mechanisms behind the microalgae accumulating these molecules require further elucidation. Recently, we have reported that the target of rapamycin (TOR)-signaling is a major pathway to regulate floridean starch synthesis by changing the phosphorylation status of CmGLG1, a glycogenin generally required for the initiation of starch/glycogen synthesis, in the unicellular red alga *Cyanidioschyzon merolae*. In the present study, we confirmed that another glycogenin, CmGLG2, is also involved in the floridean starch synthesis in this alga, since the CmGLG2 overexpression resulted in a two-fold higher floridean starch content in the cell. The results indicate that both glycogenin isoforms play an important role in floridean starch



synthesis in *C. merolae*, and would be a potential target for improvement of floridean starch production in microalgae.

Assessment of biomass potentials of microalgal communities in open pond raceways using mass cultivation.

PeerJ. 2020;8:e9418

Authors: Jo SW, Do JM, Na H, Hong JW, Kim IS, Yoon HS

Abstract

Metagenome studies have provided us with insights into the complex interactions of microorganisms with their environments and hosts. Few studies have focused on microalgae-associated metagenomes, and no study has addressed aquatic microalgae and their bacterial communities in open pond raceways (OPRs). This study explored the possibility of using microalgal biomasses from OPRs for biodiesel and biofertilizer production. The fatty acid profiles of the biomasses and the physical and chemical properties of derived fuels were evaluated. In addition, the phenotype-based environmental adaptation ability of soybean plants was assessed. The growth rate, biomass, and lipid productivity of microalgae were also examined during mass cultivation from April to November 2017. Metagenomics analysis using MiSeq identified ~127 eukaryotic phylotypes following mass cultivation with (OPR 1) or without (OPR 3) a semitransparent film. Of these, ~80 phylotypes were found in both OPRs, while 23 and 24 phylotypes were identified in OPRs 1 and 3, respectively. The phylotypes belonged to various genera, such as *Desmodesmus*, *Pseudopediastrum*, *Tetradasmus*, and *Chlorella*, of which, the dominant microalgal species was *Desmodesmus* sp. On average, OPRs 1 and 3 produced ~8.6 and 9.9 g m⁻² d⁻¹ (0.307 and 0.309 DW L⁻¹) of total biomass, respectively, of which 14.0 and 13.3 wt% respectively, was lipid content. Fatty acid profiling revealed that total saturated fatty acids (mainly C16:0) of biodiesel obtained from the microalgal biomasses in OPRs 1 and 3 were 34.93% and 32.85%, respectively; total monounsaturated fatty acids (C16:1 and C18:1) were 32.40% and 31.64%, respectively; and polyunsaturated fatty acids (including C18:3) were 32.68% and 35.50%, respectively. Fuel properties determined by empirical equations were within the limits of biodiesel standards ASTM D6751 and EN 14214. Culture solutions with or without microalgal biomasses enhanced the environmental adaptation ability of soybean plants, increasing their seed production. Therefore, microalgal biomass produced through mass cultivation is excellent feedstock for producing high-quality biodiesel and biofertilizer.

Bio-electrochemical COD removal for energy-efficient, maximum and robust nitrogen recovery from urine through membrane aerated nitrification.

Water Res. 2020 Jul 23;185:116223

Authors: De Paepe J, De Paepe K, Gòdia F, Rabaey K, Vlaeminck SE, Clauwaert P



Abstract

Resource recovery from source-separated urine can shorten nutrient cycles on Earth and is essential in regenerative life support systems for deep-space exploration. In this study, a robust two-stage, energy-efficient, gravity-independent urine treatment system was developed to transform fresh real human urine into a stable nutrient solution. In the first stage, up to 85% of the COD was removed in a microbial electrolysis cell (MEC), converting part of the energy in organic compounds (27-46%) into hydrogen gas and enabling full nitrogen recovery by preventing nitrogen losses through denitrification in the second stage. Besides COD removal, all urea was hydrolysed in the MEC, resulting in a stream rich in ammoniacal nitrogen and alkalinity, and low in COD. This stream was fed into a membrane-aerated biofilm reactor (MABR) in order to convert the volatile and toxic ammoniacal nitrogen to non-volatile nitrate by nitrification. Bio-electrochemical pre-treatment allowed to recover all nitrogen as nitrate in the MABR at a bulk-phase dissolved oxygen level below 0.1 mg O₂ L⁻¹. In contrast, feeding the MABR directly with raw urine (omitting the first stage), at the same nitrogen loading rate, resulted in nitrogen loss (18%) due to denitrification. The MEC and MABR were characterised by very distinct and diverse microbial communities. While (strictly) anaerobic genera, such as *Geobacter* (electroactive bacteria), *Thiopseudomonas*, a *Lentimicrobiaceae* member, *Alcaligenes* and *Proteiniphilum* prevailed in the MEC, the MABR was dominated by aerobic genera, including *Nitrosomonas* (a known ammonium oxidiser), *Moheibacter* and *Gordonia*. The two-stage approach yielded a stable nitrate-rich, COD-low nutrient solution, suitable for plant and microalgae cultivation.

Physiological response and oxidative transformation of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) by a *Chlorella* isolate.

Sci Total Environ. 2020 Jul 14;744:140869

Authors: Deng D, Chen HX, Wong YS, Tam NFY

Abstract

Polybrominated diphenyl ethers (PBDEs) are ubiquitous, toxic and persistent pollutants in environments. Microalgae frequent exposed to these pollutants may possess defense mechanisms against their toxicity and have the ability to metabolize them, thus are important in bioremediation. This study investigated the mechanism of a *Chlorella* isolate to degrade BDE-47, a common PBDE congener, and its subcellular responses to BDE-47 stress. Results showed that 86-98% of the spiked BDE-47 was removed by *Chlorella* via adsorption, uptake and metabolism. BDE-47 was metabolized through debromination, hydroxylation and methoxylation. The oxidative transformation to hydroxylated products was the initial and main metabolic process. BDE-47 induced the production of hydrogen peroxide (H₂O₂) in cell wall, plasma membrane and chloroplast of *Chlorella*, and such increase was regulated by nicotinamide adenine dinucleotide phosphate oxidase and H₂O₂-producing peroxidases (PODs). The activity of H₂O₂-consuming PODs and the content of glutathione were also significantly enhanced to detoxify the oxidative stress.



Isoprostanoid Profiling of Marine Microalgae.

Biomolecules. 2020 07 18;10(7):

Authors: Vigor C, Oger C, Reversat G, Rocher A, Zhou B, Linares-Maurizi A, Guy A, Bultel-Poncé V, Galano JM, Vercauteren J, Durand T, Potin P, Tonon T, Leblanc C

Abstract

Algae result from a complex evolutionary history that shapes their metabolic network. For example, these organisms can synthesize different polyunsaturated fatty acids, such as those found in land plants and oily fish. Due to the presence of numerous double-bonds, such molecules can be oxidized nonenzymatically, and this results in the biosynthesis of high-value bioactive metabolites named isoprostanoids. So far, there have been only a few studies reporting isoprostanoid productions in algae. To fill this gap, the current investigation aimed at profiling isoprostanoids by liquid chromatography -mass spectrometry/mass spectrometry (LC-MS/MS) in four marine microalgae. A good correlation was observed between the most abundant polyunsaturated fatty acids (PUFAs) produced by the investigated microalgal species and their isoprostanoid profiles. No significant variations in the content of oxidized derivatives were observed for *Rhodomonas salina* and *Chaetoceros gracilis* under copper stress, whereas increases in the production of C18-, C20- and C22-derived isoprostanoids were monitored in *Tisochrysis lutea* and *Phaeodactylum tricornutum*. In the presence of hydrogen peroxide, no significant changes were observed for *C. gracilis* and for *T. lutea*, while variations were monitored for the other two algae. This study paves the way to further studying the physiological roles of isoprostanoids in marine microalgae and exploring these organisms as bioresources for isoprostanoid production.

Astaxanthin biosynthesis promotion with pH shock in the green microalga, *Haematococcus lacustris*.

Bioresour Technol. 2020 Oct;314:123725

Authors: Han SI, Chang SH, Lee C, Jeon MS, Heo YM, Kim S, Choi YE

Abstract

In this study, the use of pH shock to improve astaxanthin synthesis in *Haematococcus lacustris* was investigated. It has been found that pH shock (pH = 4.5, 60 s) imposes stress in the cells and induces physiological changes, which result in astaxanthin accumulation. The optimal acid-base combination of pH shock was H₂SO₄-KOH, which increased the astaxanthin content per cell to $39 \pm 6.92\%$ than those of the control. In addition, pH shock can be applied simultaneously with the other inductive strategies such as high irradiance and carbon source supply. When high irradiance was applied simultaneously with pH shock, astaxanthin yield was increased $65 \pm 0.541\%$ than control. In addition, astaxanthin content per cell was increased $105 \pm 6.66\%$ than those of the control, with the concomitant application of carbon source



addition with pH shock. Herein, these novel findings provide a useful technique for producing astaxanthin using *H. lacustris*.

A proof of concept study for wastewater reuse using bioelectrochemical processes combined with complementary post-treatment technologies.

Environ Sci (Camb). 2019 Jun 24;5:1489-1498

Authors: Khan W, Nam JY, Woo H, Ryu H, Kim S, Maeng SK, Kim HC

Abstract

This article describes a proof-of-concept study designed for the reuse of wastewater using microbial electrochemical cells (MECs) combined with complementary post-treatment technologies. This study mainly focused on how the integrated approach works effectively for wastewater reuse. In this study, microalgae and ultraviolet C (UVC) light were used for advanced wastewater treatment to achieve site-specific treatment goals such as agricultural reuse and aquifer recharge. The bio-electrosynthesis of H₂O₂ in MECs was carried out based on a novel concept to integrate with UVC, especially for roust removal of trace organic compounds (TOrcs) resistant to biodegradation, and the algal treatment was configured for nutrient removal from MEC effluent. UVC irradiation has also proven to be an effective disinfectant for bacteria, protozoa, and viruses in water. The average energy consumption rate for MECs fed acetate-based synthetic wastewater was 0.28±0.01 kWh per kg of H₂O₂, which was significantly more efficient than are conventional electrochemical processes. MECs achieved 89±2% removal of carbonaceous organic matter (measured as chemical oxygen demand) in the wastewater (anolyte) and concurrent production of H₂O₂ up to 222±11 mg L⁻¹ in the tapwater (catholyte). The nutrients (N and P) remaining after MECs were successfully removed by subsequent phycoremediation with microalgae when aerated (5% CO₂, v/v) in the light. This complied with discharge permits that limit N to 20 mg L⁻¹ and P to 0.5 mg L⁻¹ in the effluent. H₂O₂ produced on site was used to mediate photolytic oxidation with UVC light for degradation of recalcitrant TOrcs in the algal-treated wastewater. Carbamazepine was used as a model compound and was almost completely removed with an added 10 mg L⁻¹ of H₂O₂ at a UVC dose of 1000 mJ cm⁻². These results should not be generalized, but critically discussed, because of the limitations of using synthetic wastewater.

Bioenergy from biofuel residues and waste.

Water Environ Res. 2020 Jun 23;:

Authors: Sheehan NP, Ng A, Murray K, Martinez E, Quell K, Ouellette C, Flagg T, Boyle J

Abstract

This article is a review of the scientific literature published in 2019 on topics relating to



bioenergy from biofuel residues and waste. This literature review is divided into the following sections: Feedstocks, Biodiesel, Bioethanol, Hydrogen, Biohydrogen, Biofuel Residues, Microalgae, and Lignocelluloses.

Mixotrophic cultivation of Chlorella for biomass production by using pH-stat culture medium: Glucose-Acetate-Phosphorus (GAP).

Bioresour Technol. 2020 Oct;313:123506

Authors: Xie Z, Lin W, Liu J, Luo J

Abstract

Here the study designed a pH-stat culture medium that named as Glucose-Acetate-Phosphorus (GAP) for the mixotrophic cultivation of Chlorella for biomass production. With no addition of pH buffer, the culture pH during mixotrophic growth was effectively maintained steady between 7.5 and 8.5 by balancing the ammonium, acetate and glucose uptakes. Based on the GAP medium supplying with 2 g·L⁻¹ of total organic carbon, the biomass productions of four Chlorella species were determined as 4.08-4.56 g·L⁻¹. In contrast to the cultivation using medium Tris-Acetate-Phosphorus (TAP), a algal culture medium that usually regarded as specific for mixotrophy, the cultivation in GAP were about 1.79-1.86 times higher in biomass production and 83.9-88.9% lower in production cost. The developed GAP medium is a promising alternative for the mixotrophic cultivation of microalgae to produce biomass and cellular contents.

Algae-Bacteria Consortia as a Strategy to Enhance H₂ Production.

Cells. 2020 May 29;9(6):

Authors: Fakhimi N, Gonzalez-Ballester D, Fernández E, Galván A, Dubini A

Abstract

Biological hydrogen production by microalgae is a potential sustainable, renewable and clean source of energy. However, many barriers limiting photohydrogen production in these microorganisms remain unsolved. In order to explore this potential and make biohydrogen industrially affordable, the unicellular microalga Chlamydomonas reinhardtii is used as a model system to solve barriers and identify new approaches that can improve hydrogen production. Recently, Chlamydomonas-bacteria consortia have opened a new window to improve biohydrogen production. In this study, we review the different consortia that have been successfully employed and analyze the factors that could be behind the improved H₂ production.



Purple phototrophic bacteria for resource recovery: Challenges and opportunities.

Biotechnol Adv. 2020 Nov 01;43:107567

Authors: Capson-Tojo G, Batstone DJ, Grassino M, Vlaeminck SE, Puyol D, Verstraete W, Kleerebezem R, Oehmen A, Ghimire A, Pikaar I, Lema JM, Hülsen T

Abstract

Sustainable development is driving a rapid focus shift in the wastewater and organic waste treatment sectors, from a "removal and disposal" approach towards the recovery and reuse of water, energy and materials (e.g. carbon or nutrients). Purple phototrophic bacteria (PPB) are receiving increasing attention due to their capability of growing photoheterotrophically under anaerobic conditions. Using light as energy source, PPB can simultaneously assimilate carbon and nutrients at high efficiencies (with biomass yields close to unity ($1 \text{ g COD}_{\text{biomass}} \cdot \text{g COD}_{\text{removed}}^{-1}$)), facilitating the maximum recovery of these resources as different value-added products. The effective use of infrared light enables selective PPB enrichment in non-sterile conditions, without competition with other phototrophs such as microalgae if ultraviolet-visible wavelengths are filtered. This review reunites results systematically gathered from over 177 scientific articles, aiming at producing generalized conclusions. The most critical aspects of PPB-based production and valorisation processes are addressed, including: (i) the identification of the main challenges and potentials of different growth strategies, (ii) a critical analysis of the production of value-added compounds, (iii) a comparison of the different value-added products, (iv) insights into the general challenges and opportunities and (v) recommendations for future research and development towards practical implementation. To date, most of the work has not been executed under real-life conditions, relevant for full-scale application. With the savings in wastewater discharge due to removal of organics, nitrogen and phosphorus as an important economic driver, priorities must go to using PPB-enriched cultures and real waste matrices. The costs associated with artificial illumination, followed by centrifugal harvesting/dewatering and drying, are estimated to be 1.9, 0.3-2.2 and 0.1-0.3 \$·kgdry biomass⁻¹. At present, these costs are likely to exceed revenues. Future research efforts must be carried out outdoors, using sunlight as energy source. The growth of bulk biomass on relatively clean wastewater streams (e.g. from food processing) and its utilization as a protein-rich feed (e.g. to replace fishmeal, 1.5-2.0 \$·kg⁻¹) appears as a promising valorisation route.

On the pyrolysis of different microalgae species in a conical spouted bed reactor: Bio-fuel yields and characterization.

Bioresour Technol. 2020 Sep;311:123561

Authors: Azizi K, Keshavarz Moraveji M, Arregi A, Amutio M, Lopez G, Olazar M

Abstract

The aim of this work was to study fast pyrolysis of three microalgae species in a continuous bench-scale conical spouted bed reactor at 500 °C. Bio-gas, bio-oil and bio-char yields have been determined and characterized by using GC, GC/MS, elemental analyzer and SEM. Bio-oil



was the main product obtained through pyrolysis of microalgae. The non-condensable gaseous stream is made up of mainly hydrogen, carbon monoxide and carbon dioxide, apart from other light hydrocarbons detected in lower concentration, as are methane, ethane, ethylene, propane and propylene. The compounds identified in the bio-oil have been categorized into hydrocarbons, nitrogen containing compounds, ketones, alcohols, acids, lactones, phenols and aldehydes. The nitrogen and carbon contents of the microalgae bio-chars are higher than those for bio-chars derived from other biomasses. Pyrolysis improved the morphology and porous structure of microalgae. Finally, the mechanism involving microalgae pyrolysis has been approached and the main reaction pathways have been proposed.

Sulfonamides-induced oxidative stress in freshwater microalga *Chlorella vulgaris*: Evaluation of growth, photosynthesis, antioxidants, ultrastructure, and nucleic acids.

Sci Rep. 2020 May 19;10(1):8243

Authors: Chen S, Wang L, Feng W, Yuan M, Li J, Xu H, Zheng X, Zhang W

Abstract

Sulfadiazine (SD), sulfamerazine (SM1), and sulfamethazine (SM2) are widely used and disorderly discharged into surface water, causing contamination of lakes and rivers. However, microalgae are regarded as a potential resource to alleviate and degrade antibiotic pollution. The physiological changes of *Chlorella vulgaris* in the presence of three sulfonamides (SAs) with varying numbers of -CH₃ groups and its SA-removal efficiency were investigated following a 7-day exposure experiment. Our results showed that the growth inhibitory effect of SD (7.9-22.6%), SM1 (7.2-45.9%), and SM2 (10.3-44%) resulted in increased proteins and decreased soluble sugars. Oxidative stress caused an increase in superoxide dismutase and glutathione reductase levels but decreased catalase level. The antioxidant responses were insufficient to cope-up with reactive oxygen species (hydrogen peroxide and superoxide anion) levels and prevent oxidative damage (malondialdehyde level). The ultrastructure and DNA of SA-treated algal cells were affected, as evident from the considerable changes in the cell wall, chloroplast, and mitochondrion, and DNA migration. *C. vulgaris*-mediated was able to remove up to 29% of SD, 16% of SM1, and 15% of SM2. Our results suggest that certain concentrations of specific antibiotics may induce algal growth, and algal-mediated biodegradation process can accelerate the removal of antibiotic contamination.

Adsorption of Pb²⁺ onto freeze-dried microalgae and environmental risk assessment.

J Environ Manage. 2020 Jul 01;265:110472

Authors: Sun X, Huang H, Zhao D, Lin J, Gao P, Yao L



Abstract

Dry microalgae *Spirulina platensis* shows a high capacity for heavy metal uptake, but there is a concern about dissolved organic carbon (DOC) residue, which is the precursor of disinfection by-products (DBPs). Vsp, a kind of *Spirulina platensis* powder prepared by vacuum freeze-drying, and Osp, a kind of *Spirulina platensis* powder prepared by the conventional oven drying-pulverization method, were subjected to assessments of their adsorption potential for Pb²⁺ and DOC residue. The adsorption mechanism of Pb²⁺ by the two adsorbents was studied by SEM, FT-IR, EDX and N₂-BET. The effects of pH, adsorbent dosage, initial Pb²⁺ concentration and contact time on the biosorption process were investigated. The results showed that Pb²⁺ biosorption by Vsp and Osp were fit well by a pseudo-second-order kinetic model and the Langmuir model. The maximum amount of Pb²⁺ biosorption by Vsp was 253 mg/g, which was 33 mg/g greater than that of Osp. In comparison with Osp, Vsp reached adsorption saturation 8 h earlier and had a remarkable effect on the control of DOC residue in water. When both adsorption capacity and environmental risks were considered, it was determined that the dosage of 0.5 g/L Vsp for 2 h of contact time was the best method, with 85.89 mg/g of Pb²⁺ removal and 3.45 mg/L of DOC residue. In summary, Vsp is a highly efficient and environmentally friendly biosorbent that can be used for heavy metal removal from water.

Autotrophic granulation of hydrogen consumer denitrifiers and microalgae for nitrate removal from drinking water resources at different hydraulic retention times.

J Environ Manage. 2020 Aug 15;268:110674

Authors: Rezvani F, Sarrafzadeh MH

Abstract

To avoid hydrogen injection and to enhance the settleability of microbial biomass in biological treatment of nitrate-contaminated drinking water resources, a new method based on granulation of a mixture of hydrogen consumer denitrifiers (HCD) and microalgae is introduced. Decreasing hydraulic retention time (HRT) was applied as the selection pressure in an up-flow photobioreactor to increase the speed of granulation and nitrate removal under autotrophic condition during a 50-day operation. Formation of granules occurred at three phases including granule nucleation, growth of granule, and mature granule, with decreasing the values of ζ -potential from -19 mV to -4 mV. Enhancement of microbial attachment within granule formation could reduce the presence of total suspended solids in the effluent. Developed granules of HCD and microalgae could settle down with velocity of 40 ± 0.6 m/h when reaching the average size of 1.2 mm at day 40. Complete NO₃⁻-N removal from drinking water was achieved from the initial stage of granulation until the end of operation at all HRTs of 3 days-5 h. The clear treated water was obtained at the growth phase when the chemical oxygen demand and phosphate were undetectable. Therefore, the application of HCD-microalgae granule is a promising way for nitrate removal from water.



Hydrothermal liquefaction of *Scenedesmus obliquus* using a novel catalyst derived from clam shells: Solid residue as catalyst for hydrogen production.

Bioresour Technol. 2020 Aug;310:123443

Authors: Arun J, Gopinath KP, SundarRajan P, Malolan R, Adithya S, Sai Jayaraman R, Srinivaasan Ajay P

Abstract

This study explores the catalytic application of waste clam shell in hydrothermal liquefaction (HTL) of microalgae (*Scenedesmus obliquus*) for liquid hydrocarbons production. Novel catalyst (calcium hydroxide) was derived from clam shells. Catalytic HTL was performed at varying temperature of 240-320 °C for catalyst load (0.2-1 wt%) at a reaction time of 60 min. Bio-oil yield was maximum (39.6 wt%) at a temperature of 300 °C for catalyst load of 0.6 wt% at a reaction time of 60 min with calorific value of 35.01 MJ/kg. Compounds like phenols, aromatic hydrocarbons, acids and aldehydes were detected in bio-oil through Gas Chromatography Mass Spectrophotometry (GC-MS). Gasification of microalgae with waste solid residue obtained from HTL was carried out for hydrogen production. Valuable hydrogen gas production was maximum (37 wt%) at a temperature of 400 °C for 3 wt% of solid residue. Water-gas shift, methanation and steam reforming reactions favoured the hydrogen gas production.

Energy conservation in photosynthetic microorganisms.

J Gen Appl Microbiol. 2020 Jun 17;66(2):59-65

Authors: Okada K, Fujiwara S, Tsuzuki M

Abstract

Photosynthesis is a biological process of energy conversion from solar radiation to useful organic compounds for the photosynthetic organisms themselves. It, thereby, also plays a role of food production for almost all animals on the Earth. The utilization of photosynthesis as an artificial carbon cycle is also attracting a lot of attention regarding its benefits for human life. Hydrogen and biofuels, obtained from photosynthetic microorganisms, such as microalgae and cyanobacteria, will be promising products as energy and material resources. Considering that the efficiency of bioenergy production is insufficient to replace fossil fuels at present, techniques for the industrial utilization of photosynthesis processes need to be developed intensively. Increase in the efficiency of photosynthesis, the yields of target substances, and the growth rates of algae and cyanobacteria must be subjects for efficient industrialization. Here, we overview the whole aspect of the energy production from photosynthesis to biomass production of various photosynthetic microorganisms.



Influence of Microcystis sp. and freshwater algae on pH: Changes in their growth associated with sediment.

Environ Pollut. 2020 Aug;263(Pt B):114435

Authors: Acuña-Alonso C, Lorenzo O, Álvarez X, Cancela Á, Valero E, Sánchez Á

Abstract

Samples from two reservoirs with eutrophication problems, located in Pontevedra and Ourense (Northwestern Spain), were cultured, along with a third crop from a reservoir with no problems detected in Ourense (Northwestern Spain). The samples were grown under the same conditions (with an average temperature of 21 ± 2 °C, and a 3000 lux light intensity) in triplicate, and their growth, absorbance and pH were studied. High correlation values were obtained for pH and cellular growth ($R^2 \geq 95\%$). The water from Salas showed the greatest microalgal growth (0.15×10^6 cells/ml to 31.70×10^6 cells/ml of *Microcystis* sp. for the last day of culturing) and the greatest increase in pH (5.72-9.02). In all the cultures studied here, the main species that reproduced was *Microcystis* sp., which can produce neurotoxins and hepatotoxins. In addition, water samples were cultured with sediments of their own reservoir and with others to observe their evolution. The sediments studied in this case were rich in biotites, which can lead phosphate to be a limiting factor for phytoplankton due to the formation and sedimentation of insoluble salts of ferric phosphate. In crops grown with sediments from the Salas reservoir, actinobacteria developed which can inhibit microalgal growth. The study of the growth of cyanobacteria and possible methods of inhibiting them directly concerns the quality of water and its ecosystems, avoiding pollution and impact on ecosystems.

Valorization of waste eggshell-derived bioflocculant for harvesting *T. obliquus*: Process optimization, kinetic studies and recyclability of the spent medium for circular bioeconomy.

Bioresour Technol. 2020 Jul;307:123205

Authors: Roy M, Mohanty K

Abstract

Waste eggshell-derived bioflocculant was used for harvesting *T. obliquus* in a circular bioeconomy approach. It was found that 120 mg L⁻¹ bioflocculant can flocculate $98.62 \pm 0.43\%$ of *T. obliquus* cells within 25 min at optimal pH 4.0 and temperature 35 °C. The influence of bioflocculant concentration, pH and temperature on zeta potential was evaluated to understand the flocculation mechanism. Microscopic and FESEM-EDX images were analyzed to evaluate the microalgal structural changes. Adsorption mechanism of bioflocculant over the microalgal cells was determined by performing adsorption kinetic studies. Pseudo-second order kinetic model was a suitable fit for the data obtained from the experiments, which indicated chemisorption as the probable mechanism. The spent medium recovered after harvesting process was successfully recycled for subsequent cultivation of *T. obliquus*, thus reducing the dependency on fresh medium. The FAME composition of the biomass treated with bioflocculant was not altered.



Overproduction of the Flv3B flavodiiron, enhances the photobiological hydrogen production by the nitrogen-fixing cyanobacterium Nostoc PCC 7120.

Microb Cell Fact. 2020 Mar 10;19(1):65

Authors: Roumezi B, Avilan L, Risoul V, Brugna M, Rabouille S, Latifi A

Abstract

BACKGROUND: The ability of some photosynthetic microorganisms, particularly cyanobacteria and microalgae, to produce hydrogen (H₂) is a promising alternative for renewable, clean-energy production. However, the most recent, related studies point out that much improvement is needed for sustainable cyanobacterial-based H₂ production to become economically viable. In this study, we investigated the impact of induced O₂-consumption on H₂ photoproduction yields in the heterocyte-forming, N₂-fixing cyanobacterium Nostoc PCC7120. **RESULTS:** The flv3B gene, encoding a flavodiiron protein naturally expressed in Nostoc heterocytes, was overexpressed. Under aerobic and phototrophic growth conditions, the recombinant strain displayed a significantly higher H₂ production than the wild type. Nitrogenase activity assays indicated that flv3B overexpression did not enhance the nitrogen fixation rates. Interestingly, the transcription of the hox genes, encoding the NiFe Hox hydrogenase, was significantly elevated, as shown by the quantitative RT-PCR analyses. **CONCLUSION:** We conclude that the overproduced Flv3B protein might have enhanced O₂-consumption, thus creating conditions inducing hox genes and facilitating H₂ production. The present study clearly demonstrates the potential to use metabolic engineered cyanobacteria for photosynthesis driven H₂ production.

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.

Understanding the by-product formation potential during phenol oxidation from in-situ electro-generated radicals by microalgae harvesting.

Environ Technol. 2020 Mar 04;:1-13

Authors: Singh H, Kumar N, Mishra BK

Abstract

Advanced oxidation processes have gained colossal attention owing to the prospect of accessible mineralization, but by-product formation and its toxicity evaluation are still inconclusive. The present study demonstrated the performance of electrochemical oxidation process supported with graphite electrodes for the oxidation of phenol from modulated coke oven wastewater. The results suggested that the hydrogen peroxide along with the in-situ synthesized oxidizing agents has the ability to increase the phenol mineralization 1.5 times and by-product toxicity potential on microalgae, *Scenedesmus* sp. CBIIT(ISM) also revealed that chlorophyll-a synthesis has increased after the electro-oxidation process in coke oven wastewater. The experimental results for phenol mineralization and by-product formation were validated using a mass spectrophotometer.

Evaluation of hydrogen yield potential from *Chlorella* by photo-fermentation under diverse substrate concentration and enzyme loading.

Bioresour Technol. 2020 May;303:122956

Authors: Liu H, Zhang Z, Zhang H, Lee DJ, Zhang Q, Lu C, He C

Abstract

Chlorella is widely distributed, can be cultured in waste water and had short growth cycle. The high carbohydrate composition shows great potential for bioenergy output. In this work, concentrated *Chlorella* solution was adopted as raw material. Reducing sugar concentration, pH, and cumulative bio-hydrogen yield were taken as indexes, the effects of substrate concentration and enzyme (cellulase or neutral protease) load on photo-fermentation bio-hydrogen production process from microalgae biomass were investigated. Results showed that highest cumulative hydrogen yield was obtained at the optimal substrate concentration of 25 g/L, when the load of cellulase and protease are both 15%, the effect is the best which were 16.65 mL, 29.44 mL, and 43.62 mL, respectively. Results fitted well to the Gompertz model, indicating the feasibility of photo-fermentative bio-hydrogen production from concentrated *Chlorella*.



Po uptake in microalgae at different seawater pH: An experimental study simulating ocean acidification.

Mar Pollut Bull. 2020 Feb;151:110844

Authors: Uddin S, Bebbehani M, Al-Musallam L, Kumar VV, Sajid S

Abstract

Climate change effects such as ocean acidification (OA) are known to affect the trace metal distribution. This experimental study provides the first data on ²⁰⁹Po uptake rates and ²¹⁰Po concentration in five microalgae species under different pH scenarios. The experiment was conducted in replicates at three pH conditions 8.2, 8.0, and 7.5, representing the current and future climate change scenario as per IPCC RCP8.5. The ²⁰⁹Po uptake in the phytoplankton was highest in *Thalassiosira weissflogi*, i.e. 83% of the ²⁰⁹Po tracer was taken up at 8.2 pH whereas the lowest uptake was observed in *Dunaliella salina* equivalent to 20% at 7.5 pH. Similar behavior was observed in ²¹⁰Po concentrations in these microalgae, where ²¹⁰Po ranged between 3.16 ± 0.03 and 11.6 ± 0.04 Bq kg⁻¹ wet weight (ww), with the highest in the *Thalassiosira weissflogi* at 8.2 pH, and the lowest in *Dunaliella salina* at 7.5 pH. The difference in ²⁰⁹Po uptake and ²¹⁰Po concentration was statistically significant ($p < 0.001$) both among species and the pH treatments in the order: *Thalassiosira weissflogi* > *Tetraselmis suecica* > *Chaetoceros muelleri* > *Isochrysis galbana* > *Dunaliella salina* and 8.2 > 8.0 > 7.5. A higher concentration of ²⁰⁹Po in seawater was measured at low pH condition in all the experimental tanks. Though the data clearly show the difference in concentration and uptake of polonium at different pH conditions, it is not known if lower pH is affecting the adsorbed or absorbed fraction. A detailed investigation will be required to understand the process as it can have a significant effect on biomagnification and marine food chain transfer under changing climatic scenarios.

Toxicity of microwave-synthesized silver-reduced graphene oxide nanocomposites to the microalga *Chlorella vulgaris*: Comparison with the hydrothermal method synthesized counterparts.

J Environ Sci Health A Tox Hazard Subst Environ Eng. 2020;55(6):639-649

Authors: Nazari F, Jafarirad S, Movafeghi A, Kosari-Nasab M, Kazemi EM

Abstract

The increased applications of nanomaterials in industry and biomedicine have resulted in a rising concern about their possible toxic impacts on living organisms. It has been claimed that the phytosynthesized nanomaterials have lower toxicity in comparison to their chemically synthesized counterparts. Therefore, it is important to evaluate their toxic effects on the environment. In the present study, we investigated the toxic effects of microwave-synthesized silver-reduced graphene oxide nanocomposites (MS-Ag-rGO) on *Chlorella vulgaris*. Algal cells



were treated by 1, 2, 4 and 6 mg L⁻¹ MS-Ag-rGO for 24 h. The obtained data with three replicates were examined using analysis of variance. Analysis of different growth parameters revealed that MS-Ag-rGO possessed significant dose-dependent toxic effect on *C. vulgaris*. Scanning electron microscope and fluorescence microscope images of the treated cells established morphological shrinkages and alteration in position of nucleoli. Moreover, reduction in the phenol and flavonoid contents, enhancement of H₂O₂ content, changes in the antioxidant enzymes activity and decreases in the growth parameters as well as photosynthetic pigments quantities confirmed the toxicity of MS-Ag-rGO to the *C. vulgaris* cells. Our findings revealed that MS-Ag-rGO possessed higher toxicity on *C. vulgaris* than Ag-rGO synthesized by hydrothermal technique.

Microalgae carbon fixation integrated with organic matters recycling from soybean wastewater: Effect of pH on the performance of hybrid system.

Chemosphere. 2020 Jun;248:126094

Authors: Song C, Han X, Qiu Y, Liu Z, Li S, Kitamura Y

Abstract

Microalgae have been considered as promising alternative for CO₂ fixation and wastewater purification. In our previous work, a hybrid microalgae CO₂ fixation concept has been put forward, which initially used carbonate solution absorb CO₂, and then provided obtained bicarbonate as nutrition for microalgae growth to avoid the challenge of low CO₂ solubility and carbon fixation efficiency in the conventional process. In this work, the proposed hybrid system was further intensified via integrating soybean wastewater nutrition removal with bicarbonate-carbon (NH₄HCO₃ and KHCO₃) conversion. The investigation results indicated that the maximum biomass productivity (0.74 g L⁻¹) and carbon bioconversion efficiency (46.9%) were achieved in low-NH₄HCO₃ concentration system with pH adjusted to 7. pH adjustment of different bicarbonate systems also enhanced total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) removal efficiency up to 87.5%, 99.5% and 77.6%, respectively. In addition, maximum neutral lipid (14.4 mg L⁻¹·d⁻¹) and polysaccharide (14.5 mg L⁻¹·d⁻¹) productivities could be obtained in the KHCO₃ systems, while higher crude protein productivity (48.1 mg L⁻¹·d⁻¹) was yielded in the NH₄HCO₃ systems.

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% H₂SO₄) 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.

Co-Hydrothermal gasification of *Chlorella vulgaris* and hydrochar: The effects of waste-to-solid biofuel production and blending concentration on biogas generation.

Bioresour Technol. 2020 Apr;302:122793

Authors: Sztancs G, Juhasz L, Nagy BJ, Nemeth A, Selim A, Andre A, Toth AJ, Mizsey P, Fozér D

Abstract

This study investigates enhanced biogas production via co-Hydrothermal gasification (co-HTG) of wet *Chlorella vulgaris* biomass and hydrochar (HC). Hydrothermal carbonization was applied to valorize struvite containing waste microalgae stream into solid bio-fuel with improved combustion properties. The effects of HC quality and mixing ratio are investigated on biogas yield, composition and carbon conversion ratio. The results show that the application of blending components promotes H₂, CH₄ formation and selectivity in hydrothermal gasification. The total co-HTG gas yield is increased from 19.13 to 46.95 mol kg⁻¹ at 650 °C and 300 bar by applying 5 wt% HC blending concentration and reduced level of volatile matter content (24.61 wt%). The obtained high hydrogen, methane yields and carbon conversion ratio (19.49, 2.98 mol kg⁻¹, 82.31%, respectively) indicate effective hydrothermal upgrading potentials in case of wet and waste biomass feedstocks.

Pretreatment of microalgal biomass for efficient biohydrogen production - Recent insights and future perspectives.

Bioresour Technol. 2020 Apr;302:122871

Authors: Nagarajan D, Chang JS, Lee DJ



Abstract

Biohydrogen is a plausible alternative fuel solution for the contemporary issues regarding global warming and the steadily increasing greenhouse gas emissions, because of its high energy content and carbon-free combustion properties. Hydrogen does not exist in its natural state and the current hydrogen production technologies (steam methane reforming, water splitting) are energy-intensive, accompanied by a huge carbon footprint. Dark fermentative hydrogen production by anaerobic hydrogen-producing bacteria is a green, sustainable and emission-free pathway for hydrogen production. Microalgal biomass is considered as the third generation biofuel feedstock and is receiving academic and industrial research attention for its carbon sequestration abilities. This review discusses in detail about the pretreatment methods that could be adapted for microalgal biomass for effective biohydrogen production. Microalgal cell wall structure and the associated polymeric carbohydrates that offer certain recalcitrance are critically analyzed and future research perspectives are presented.

Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis.

Bioresour Technol. 2020 Apr;302:122822

Authors: Rajesh Banu J, Preethi, Kavitha S, Gunasekaran M, Kumar G

Abstract

Microalgae are source of third generation biofuel having the key advantage of high lipid productivity. In recent times, biorefinery is seen as promising option to further reduce the production cost of microalgae biofuel. However, exact energy balance analysis has not been performed on important biorefinery routes. In this aspect, three biorefinery routes, all based on lipid based biorefinery route are evaluated for economical production of microalgal biofuel and valorised products. Biorefinery route 1 involves production of biodiesel, pigments, and animal feed. Biorefinery route 2 involves biogas and pigments production and two stage fermentation, and third biorefinery route involves bio-hydrogen and pigments production. Finally, the technoeconomic assessment of three biorefinery routes were reviewed, net energy savings, and life-cycle costing approaches to economize microalgal biorefinery are suggested.

Temperate-zone cultivation of Oedogonium in municipal wastewater effluent to produce cellulose and oxygen.

J Ind Microbiol Biotechnol. 2020 Feb;47(2):251-262

Authors: Piotrowski MJ, Graham LE, Graham JM

Abstract

Cultivation of the filamentous chlorophyte Oedogonium in municipal wastewater effluent is known to improve water quality and yield lipid- and protein-rich biomass for industrial



applications. Chlorophyte celluloses, whose molecular organization and physical traits differ from those of plants, represent yet another valuable extractive, and algal oxygen production is of economic value in wastewater treatment. Consequently, we explored cellulose and oxygen production from *Oedogonium* biomass batch-cultivated in treated secondary municipal wastewater effluent. We compared biomass, cellulose, and oxygen production outside and within an adjacent greenhouse, under differing dissolved CO₂ and pH conditions, and during temperate-zone seasonal change from summer through fall. Overall production did not differ within or outside the greenhouse, but outside production was higher in summer and lower in fall as air temperatures declined. Batch cultivation offered advantages, but high levels of mixing and CO₂ were essential to maintain neutral pH for optimal algal growth and oxygen production.

Conversion and Stability of New Metabolites of Paralytic Shellfish Toxins under Different Temperature and pH Conditions.

J Agric Food Chem. 2020 Feb 05;68(5):1427-1435

Authors: Che Y, Ding L, Qiu J, Ji Y, Li A

Abstract

A number of new C-11 hydroxyl metabolites (so-called M-toxins) of paralytic shellfish toxins (PSTs) have been discovered in contaminated shellfish, and trace amounts have also been detected in some strains of PST-producing microalgae. To investigate the chemical conversion and stability of M-toxins, mussel extracts were purified with solid-phase extraction cartridges (Oasis HLB) and Biogel P-2 resin columns and four partially purified M-toxin fractions were stored at different temperatures (-20, 4, and 20 °C) and pH values (3, 4, and 5). The concentrations and profiles of M-toxins in these fractions were analyzed using liquid chromatography coupled with tandem mass spectrometry for 27 weeks. Results further confirmed the chemical conversion pathway M1 → M3 → M5 and determined for the first time two new transformation pathways: M2 → M4 → M6 and neosaxitoxin (NEO) → M10. The half-lives of M1, M2, M4, and M10 were calculated using a first-order degradation kinetics model, which indicated that the degradation of all M-toxins was dependent upon the temperature and pH, increasing with rising temperature and pH. In comparison to M4 and M10, M1 was more sensitive to the temperature, followed by M2. Results suggest that M-toxins should be maintained at a low temperature (-20 °C) and low pH (3) for their prolonged storage. M-toxins were less stable than all of the common analogues of PSTs, which may be beneficial for shellfish to achieve rapid detoxification through transformation of PSTs to M-toxins. These new findings are of significance because they enable further understanding of the metabolism of PSTs and their detoxification mechanisms in contaminated shellfish.

Photoautotrophic cultures of *Chlamydomonas reinhardtii*: sulfur deficiency, anoxia, and hydrogen production.

Photosynth Res. 2020 Mar;143(3):275-286



Abstract

The aim of this work was a comparative study of S-repleted and S-depleted photoautotrophic cultures of *Chlamydomonas reinhardtii* under aerobic and anoxic conditions with the main focus on PSII activity. For that we used photobioreactor with short light path connected on-line to PAM fluorometer and cultivated microalgae in twice concentrated HS medium to avoid any uncontrolled limitation by mineral elements. Photoautotrophic cultures grown under Ar + CO₂ gas mixture did not reach the same Chl (a + b) concentration as control culture (grown under air + CO₂). At pO₂ 40% of air saturation (96 μM O₂), the actual quantum yield of PSII started to decrease. Under microaerobic conditions when cultures stopped growing, the most significant changes in PSII function were observed. Maximum quantum yield F_v/F_m decreased significantly along with performance index, PI_{abs}. It was accompanied by increase of fluorescence at J point, V_j. Results indicate that microaerobic conditions are stressful for photoautotrophic cultures. Photoautotrophic cultures of microalgae under S-deprivation in aerobic or anaerobic conditions showed similar behavior as photoheterotrophic ones described earlier. However, photoautotrophic cultures during anaerobiosis establishment did not show sharp "switch off" effect of actual quantum yield. We show also that S-deprivation under air or argon as well as the growth under Ar + CO₂ cause significant increase of initial rise of fluorescence, which indicates that PSII and oxygen-evolving complex might be disintegrated.

The use of response surface methodology for improving fatty acid methyl ester profile of *Scenedesmus vacuolatus*.

Environ Sci Pollut Res Int. 2020 Aug;27(22):27457-27469

Authors: Ghosh A, Samadhiya K, Kashyap M, Anand V, Sangwan P, Bala K

Abstract

The present study has been designed to optimise certain important process parameters for *Scenedesmus vacuolatus* to achieve efficient carbon dioxide extenuation as well as suitable fatty acid profile in context to improve biodiesel properties. The effect of varying sodium bicarbonate concentration was evaluated in single and multicomponent system such as nitrate, phosphate, inoculum size to observe interactive effects on algae biomass production, carbon dioxide (CO₂) removal efficiency and fatty acid methyl ester (FAME) profile. Maximum biomass productivity of 117.0 ± 7.7 mg/L/day with 3 g/L of sodium bicarbonate was obtained i.e. approximately 2 folds higher than the control. Under multicomponent exposure, maximum biomass of 1701.5 ± 88.8 mg/L and maximum chlorophyll concentration of 15.3 ± 6.4 mg/L were achieved on 14th day at 3 g/L sodium nitrate, 0.1 g/L dipotassium hydrogen phosphate, 2 g/L of sodium bicarbonate and initial cell density of 0.3 (N3P0.1B2OD0.3). FAME content of 46.1 mg/g of biomass was obtained at this combination which is approximately 3 folds higher than the FAME content obtained under nitrogen and phosphate deprivation (16.6 mg/g at N0P0B2OD0.3). Confocal microscopy images confirmed the results with enhanced lipid droplet accumulation at high bicarbonate concentration as compared with the control. This interactive study concluded the variability in FAME profile along with the exposure to varying nutrient concentrations.



In Situ Microscopy for Real-time Determination of Single-cell Morphology in Bioprocesses.

J Vis Exp. 2019 12 05;(154):

Authors: Marbà-Ardébol AM, Emmerich J, Muthig M, Neubauer P, Junne S

Abstract

In situ monitoring in microbial bioprocesses is mostly restricted to chemical and physical properties of the medium (e.g., pH value and the dissolved oxygen concentration). Nevertheless, the morphology of cells can be a suitable indicator for optimal conditions, since it changes with dependence on the growth state, product accumulation and cell stress. Furthermore, the single-cell size distribution provides not only information about the cultivation conditions, but also about the population heterogeneity. To gain such information, a photo-optical in situ microscopy device¹ was developed to enable the monitoring of the single-cell size distribution directly in the cell suspension in bioreactors. An automated image analysis is coupled to the microscopy based on a neural network model, which is trained with user-annotated images. Several parameters, which are gained from the captures of the microscope, are correlated to process relevant features of the cells, like their metabolic activity. Until now, the presented in situ microscopy probe series was applied to measure the pellet size in filamentous fungi suspensions. It was used to distinguish the single-cell size in microalgae cultivation and relate it to lipid accumulation. The shape of cellular particles was related to budding in yeast cultures. The microscopy analysis can be generally split into three steps: (i) image acquisition, (ii) particle identification, and (iii) data analysis, respectively. All steps have to be adapted to the organism, and therefore specific annotated information is required in order to achieve reliable results. The ability to monitor changes in cell morphology directly in line or on line (in a by-pass) enables real-time values for monitoring and control, in process development as well as in production scale. If the off line data correlates with the real-time data, the current tedious off line measurements with unknown influences on the cell size become needless.

Metabolic responses of the green microalga *Dunaliella salina* to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment.

Aquat Toxicol. 2019 Dec;217:105356

Authors: Bahador E, Einali A, Azizian-Shermeh O, Sangtarash MH

Abstract

In the present study, the biochemical responses and antioxidant enzymes activity of the *Dunaliella salina*, a green microalga, to the interaction of silver nanoparticles (AgNPs) and salicylic acid (SA) were investigated. Algal suspensions in the phase of logarithmic growth were subjected to the concentrations of 0, 5, 15, and 25 pM AgNPs with or without 1 mM SA. AgNPs



level of 25 pM declined cell division but highly accumulated levels of chlorophyll, β -carotene, proteins, free amino acid, carbohydrates, and hydrogen peroxide, which was associated with enhanced the activity of proteolysis, lipid peroxidation, and antioxidant enzymes. SA-treated cells at 25 pM AgNPs improved cell growth but declined the activities of antioxidant enzymes and proteolytic along with a lower accumulation of metabolites except β -carotene relative to untreated controls. These results suggest that AgNPs treatment induce oxidative stress in *D. salina* cells, which tolerated by alga through the metabolic modifications and accumulating β -carotene, while SA induces AgNPs tolerance by the mechanisms that direct carbon flux to growth and β -carotene biosynthesis rather than the antioxidant enzymes or osmoprotectant metabolites.

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.



Amyloid protein produced by *B. cereus* CR4 possesses bioflocculant activity and has potential application in microalgae harvest.

Biotechnol Lett. 2020 Jan;42(1):79-91

Authors: Sarang MC, Nerurkar AS

Abstract

Bacillus cereus CR4 from the flocs of activated sludge was found to produce an extracellular bioflocculant, which was characterized as amyloid protein and demonstrated to have potential application in microalgae recovery. Cell surface amyloid production was demonstrated by fluorescence, confocal and scanning electron microscopy. Birefringence, spectral shift assay, TEM, FTIR and CD spectra confirmed the amyloid nature of the purified protein that demonstrated flocculation. The gene for amyloid protein of *B. cereus* CR4 was found to be related to *tasA* gene of amyloid protein produced by *Bacillus subtilis*. The results demonstrated that the amyloid protein produced by *B. cereus* CR4 possessed a novel bioflocculant activity which at pH below 4.5 reached to a maximum of 86.87%. The amyloid bioflocculant producing *B. cereus* CR4 has a potential in biotechnological application like *Scenedesmus* biomass recovery.

Development and Characterization of Astaxanthin-Containing Whey Protein-Based Nanoparticles.

Mar Drugs. 2019 Nov 04;17(11):

Authors: Zanoni F, Vakarelova M, Zoccatelli G

Abstract

Astaxanthin (ASX) is a carotenoid of great interest due to its potential health benefits. However, its use in the food, feed, and pharmaceutical fields is limited due to low bioavailability, poor stability during thermochemical treatments, susceptibility to oxidation, and poor organoleptic characteristics. The aim of this work was to develop a method to stabilize astaxanthin extracted from the microalgae *Haematococcus pluvialis* (H.p.) and to improve its nutritional and functional properties through nanoencapsulation. Nanoparticles (NPs) were produced by emulsification-solvent evaporation technique starting from H.p. oleoresin using whey proteins concentrate (WPC) as stabilizer. The efficiency of encapsulation was 96%. The particle size (Z-average) was in the range of 80-130 nm and the superficial charge (measured as zeta-potential) was negative (-20 to -30 mV). The stability of the NPs upon resuspension in water was assayed through a panel of stress tests, i.e., extreme pH, UV radiation, Fe³⁺ exposition, and heating at 65 °C, that always showed a superior performance of encapsulated ASX in comparison to oleoresin, even if NPs tended to precipitate at pH 3.5-5.5. Simulated gastroenteric digestion was conducted to study the release of ASX in physiological conditions, and showed a maximum bioaccessibility of 76%, with 75% ASX converted into the more bioavailable free form. The collected data suggest that NPs might have possible future applications as supplements for human and animal diets.



Archaea inhibition: Strategies for the enhancement of volatile fatty acids production from microalgae.

Waste Manag. 2020 Feb 01;102:222-230

Authors: Magdalena JA, González-Fernández C

Abstract

In the present study, anaerobic sludge was subjected to thermal and chemical pretreatments to favour VFAs production from a protein-rich waste (i.e. microalgae biomass). Sludge pretreatments have been previously used in hydrogen production; however, information about how they can affect VFAs production from microalgae is still lacking. Thermal pretreatment was studied at: (i) 80 °C for 10 and 30 min; (ii) 120 °C for 10 and 30 min; and (iii) 100 °C for 20 min. 2-bromoethanesulfonate (BES) at 10 mM and 30 mM was used as chemical pretreatment. Besides, a combination of both pretreatment methods (80 °C and 120 °C at 10 mM and 30 mM BES) was also tested. Thermal pretreatment increased organic matter conversions into VFAs (up to 71% COD-VFAs/COD_{in}) when compared to control values (40% in the untreated anaerobic sludge). Acetic acid was the most abundant VFAs at high temperatures (120 °C) and when BES was employed (up to 60% and 40%, respectively, in terms of COD). On the other hand, propionic acid was the most abundant product at low temperatures and in the untreated anaerobic sludge (up to 60% in terms of COD). This research work might set guidelines in order to choose a suitable sludge pretreatment for VFAs production from microalgae.

Tear Down the Fluorescent Curtain: A New Fluorescence Suppression Method for Raman Microspectroscopic Analyses.

Sci Rep. 2019 10 31;9(1):15785

Authors: Yakubovskaya E, Zaliznyak T, Martínez Martínez J, Taylor GT

Abstract

The near exponential proliferation of published Raman microspectroscopic applications over the last decade bears witness to the strengths and versatility of this technology. However, laser-induced fluorescence often severely impedes its application to biological samples. Here we report a new approach for near complete elimination of laser-induced background fluorescence in highly pigmented biological specimens (e.g., microalgae) enabling interrogation by Raman microspectroscopy. Our simple chemiphotobleaching method combines mild hydrogen peroxide oxidation with broad spectrum visible light irradiation of the entire specimen. This treatment permits observing intracellular distributions of macromolecular pools, isotopic tracers, and even viral propagation within cells previously not amenable to Raman microspectroscopic examination. Our approach demonstrates the potential for confocal Raman microspectroscopy becoming an indispensable tool to obtain spatially-resolved data on the chemical composition of highly fluorescent biological samples from individual cells to environmental samples.



Hybrid liquid biphasic system for cell disruption and simultaneous lipid extraction from microalgae *Chlorella sorokiniana* CY-1 for biofuel production.

Biotechnol Biofuels. 2019;12:252

Authors: Yew GY, Chew KW, Malek MA, Ho YC, Chen WH, Ling TC, Show PL

Abstract

Background: The extraction of lipids from microalgae requires a pretreatment process to break the cell wall and subsequent extraction processes to obtain the lipids for biofuels production. The multistep operation tends to incur high costs and are energy intensive due to longer process operations. This research work applies the combination of radicals from hydrogen peroxide with an organic solvent as a chemical pretreatment method for disrupting the cell wall of microalgae and simultaneously extracting lipids from the biomass in a one-step biphasic solution. **Result:** Several parameters which can affect the biphasic system were analyzed: contact time, volume of solvent, volume ratio, type of organic solvent, biomass amount and concentration of solvents, to extract the highest amount of lipids from microalgae. The results were optimized and up to 83.5% of lipid recovery yield and 94.6% of enhancement was successfully achieved. The results obtain from GC-FID were similar to the analysis of triglyceride lipid standard. **Conclusion:** The profound hybrid biphasic system shows great potential to radically disrupt the cell wall of microalgae and instantaneously extract lipids in a single-step approach. The lipids extracted were tested to for its comparability to biodiesel performance.

Sensory properties of aqueous dispersions of protein-rich extracts from *Chlorella protothecoides* at neutral and acidic pH.

J Sci Food Agric. 2020 Feb;100(3):1344-1349

Authors: Grossmann L, Wörner V, Hinrichs J, Weiss J

Abstract

BACKGROUND: Water-soluble proteins extracted from the heterotrophically cultivated microalga *Chlorella protothecoides* have been shown to have a good solubility over a broad pH range, which makes them a promising candidate for beverage formulations. This study investigated the sensory properties of dispersions of a protein-rich extract from *C. protothecoides* at neutral and pH 3.

RESULTS: Sensory acceptance tests of the pure extract revealed an overall low acceptance at pH 7 without sucrose addition. Sensory acceptance was significantly ($P \leq 0.05$) increased by lowering the pH to 3 with citric acid, and the addition of 50 g kg⁻¹ sucrose. Here, overall positive sensory acceptance ratings were achieved up to a protein extract concentration of 40 g kg⁻¹. Basic taste evaluations showed only low bitterness scores and no significant ($P > 0.05$) increase in bitterness with decreasing pH.

CONCLUSION: It is suggested that protein-rich extracts from *C. protothecoides* have promising sensory properties in beverage formulations. © 2019 The Authors. Journal of the Science of



Aged microplastics polyvinyl chloride interact with copper and cause oxidative stress towards microalgae *Chlorella vulgaris*.

Aquat Toxicol. 2019 Nov;216:105319

Authors: Fu D, Zhang Q, Fan Z, Qi H, Wang Z, Peng L

Abstract

Microplastics (MPs) could pose potential risks to microalgae, the primary producer of marine ecosystems. Currently, few studies focus on the interaction of aged MPs with other pollutants and their toxic effects to microalgae. Therefore, the present study aimed to investigate i) the aging of microplastics polyvinyl chloride (mPVC) in simulated seawater and the changes in physical and chemical properties; ii) the effects of single mPVC (virgin and aged) and copper on microalgae *Chlorella vulgaris*; and iii) the interaction of aged mPVC and copper and the oxidative stress towards *C. vulgaris*. In this study, some wrinkles, rough and fractured surface textures can be observed on the aged mPVC, accompanying with increased hydroxyl groups and aromatic carbon-carbon double bond but decreased carbon hydrogen bond. It was found that single virgin or aged mPVC at low concentration (10 mg/L) had significant inhibition on the growth of *C. vulgaris* but no inhibition at higher concentration (100, 1,000 mg/L), which can be reasonably explained by the aggregation and precipitation of mPVC at high concentration. The aging of mPVC inhibited the growth of *C. vulgaris* with the maximum growth inhibition ratio (IR) of 35.26% as compared with that of virgin mPVC (IR = 28.5%). However, the single copper could significantly inhibit the growth of *C. vulgaris* and the inhibitory effects increased with concentration (0.2, 0.5, 1.0 mg/L). Furthermore, both the single aged mPVC (10 mg/L) and copper (0.5 mg/L) caused serious cell damage, although the concentration of superoxide dismutase (SOD) and the intracellular malonaldehyde (MDA) increased. In contrast to single treatment, the growth of *C. vulgaris* can be enhanced by the combined group with copper (0.5 mg/L) and aged mPVC (10 mg/L).

Combinational system for biodegradation of olive oil mill wastewater phenolics and high yield of bio-hydrogen production.

J Biotechnol. 2019 Dec 20;306:47-53

Authors: Papazi A, Pappas I, Kotzabasis K

Abstract

Olive oil mill wastewater (OMW) is a significant pollutant in the Mediterranean region. In the present contribution, we showed clearly that microorganisms (microalgae and OMW-microflora) activated the biodegradation of OMW-phenolics and produced a high yield of hydrogen (H₂). In



a closed incubation system, the appropriate adjustment of OMW-pH leads to the establishment of anoxic conditions through the oxygen consumption of microorganisms during the first incubation day. The biodegradation procedure of OMW-phenolics needs oxygen. Therefore, after the establishment of anoxic conditions, the biodegradation stopped and the activation of hydrogenases started, leading to a continuous high yield of bio-hydrogen production. If the cultivation system re-opened (oxygen enrichment), the OMW-phenolic biodegradation (oxygen dependent process) started again and therefore the detoxified OMW could be used for further biotechnological applications (production of biodiesel, bioalcohols, organic fertilizers, etc.). Apart from the environmental compatibility of the method and the sustainability of such a combinational application (OMW detoxification and high yield of hydrogen production) in the context of a green biotechnology approach, the cost/profit ratio appears to be particularly tempting and guarantees its widespread use in the near future. The present contribution proposes a solution to a major environmental problem by upgrading its solution to a high-value product.

Effects of *Chlorella vulgaris* polysaccharides accumulation on growth characteristics of *Trachemys scripta elegans*.

Int J Biol Macromol. 2019 Dec 01;141:1304-1313

Authors: Gui J, Tong W, Huang S, Liang X, Fang Z, Wang W, Zhang Y

Abstract

The present study investigated the effects of the accumulated polysaccharides in *Chlorella vulgaris* microalgae on the growth characteristics of *Trachemys scripta elegans*. Sodium alginate was used to prepare immobilized *C. vulgaris*, and the antioxidant effects of the accumulated polysaccharides in it were determined using *Caenorhabditis elegans* as a model. We determined the specific growth rates of *T. s. elegans* (10 in each group) and their levels of non-specific immune-related indexes (including alkaline phosphatase; total superoxide dismutase; catalase; malondialdehyde). Under optimal culturing conditions, the accumulated polysaccharide content in *C. vulgaris* reached 32.7% (dry weight). Polysaccharides from *C. vulgaris* significantly improved the hydrogen peroxide-induced oxidative stress resistance and resulted in the enhancement of stress resistance-related antioxidant enzymes, including total superoxide dismutase and catalase ($p < 0.05$). The accumulated polysaccharides in *C. vulgaris* were heteropolysaccharides comprising rhamnose, ribose, arabinose, xylose, 2-deoxy-D-glucose, mannose, glucose, galactose, and glucosamine with a molar ratio of 0.26: 0.62: 0.21: 0.10: 0.08: 0.18: 1.00: 0.42: 0.17. Compared with the control group with common feeds, suspended and immobilized *C. vulgaris* with higher accumulated polysaccharide levels had a positive effect on the specific growth rate of the *T. s. elegans* ($p < 0.05$). Further, the suspended and immobilized *C. vulgaris* with higher accumulated polysaccharide levels significantly increased serum alkaline phosphatase, total superoxide dismutase and catalase activity ($p < 0.05$) and decreased serum malondialdehyde levels of *T. s. elegans* ($p < 0.05$).



Adsorption thermodynamic characteristics of *Chlorella vulgaris* with organic polymer adsorbent cationic starch: Effect of temperature on adsorption capacity and rate.

Bioresour Technol. 2019 Dec;293:122056

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

Abstract

Aiming at optimizing the adsorption process of *Chlorella vulgaris* and cationic starch, the adsorption thermodynamic characteristics were evaluated. Different from inorganic calcium salt adsorbent, the adsorption nature of organic polymer cationic starch is exothermic ($\Delta H^\circ < 0$) and spontaneous ($\Delta G^\circ < 0$). Besides, the adsorption capacity and rate can be well described by Langmuir isotherm and pseudo second kinetic models. As results of exothermic nature and great driving force of lower temperature, the adsorption capacity and rate declined with the rising temperature. The maximal values of them were obtained at 278.15K, which were 9148.14 mg microalgae (g cationic starch)⁻¹ and 8.74×10^{-6} mg g⁻¹ min⁻¹. Additionally, with insufficient adsorbent, the highest adsorption efficiency (96.37%) was achieved at 278.15K for stirring 150 min. For 288.15, 298.15, 308.15 and 318.15K, the adsorption efficiency decreased to 93.77%, 86.75%, 83.32% and 81.57% and the time consumed were at least 40 min longer.

Effects of carbon concentration, pH, and bubbling depth on carbon dioxide absorption ratio in microalgae medium.

Environ Sci Pollut Res Int. 2019 Nov;26(32):32902-32910

Authors: Yin D, Wang Z, Wen X, Ding Y, Hou X, Geng Y, Li Y

Abstract

The microalgae-based CO₂ sequestration is considered to be an effective technique with great potential to cope with carbon emission. However, most researches are only focused on microalgae; the effects of physicochemical factors, which are carbon concentration, medium pH, and bubbling depth, on absorption and utilization of supplied CO₂ in culture is less known. In order to understand and improve CO₂ absorption in microalgae culture, the effects of these three factors were studied with different levels and combinations. Results revealed that when medium carbon concentration increased from 4.76 to 95.24 mmol/L, CO₂ absorption ratio increased by about 12%, 10%, 12%, and 11% at medium depths of 10, 20, 40, and 80 cm, with the initial pH 10.6 to 9.7 by bubbling CO₂, respectively. As bubbling depth increased from 10 to 80 cm, CO₂ absorption ratio increased by about 25%, 22%, and 25% at carbon concentrations of 4.76, 9.52, and 95.24 mmol/L, with the initial pH 10.6 to 9.7 by bubbling CO₂, respectively. In range of 10.6-7.0, pH had no significant effect on CO₂ absorption ratio ($P > 0.05$) when carbon concentration is below 9.52 mmol/L, while above 9.52 mmol/L, pH had significant effect on CO₂ absorption ratio ($P < 0.05$). It was found for the first time that the effect of pH on the CO₂ absorption ratio was affected by carbon concentration. In addition, equilibrium pH, at which the CO₂ partial pressure in the medium equals to that in the air, of medium with different carbon concentrations was also determined. Overall, in microalgae



culture for CO₂ sequestration, increasing CO₂ bubbling depth and keeping higher carbon concentration and higher pH can improve CO₂ absorption ratio, which will optimize the biofixation of CO₂ by microalgae furthermore.

Extraction, structural and functional properties of *Haematococcus pluvialis* protein after pigment removal.

Int J Biol Macromol. 2019 Nov 01;140:1073-1083

Authors: Zhu Y, Zhao X, Zhang X, Liu H

Abstract

Protein from *Haematococcus pluvialis* (*H. pluvialis*) residues after pigment removal was prepared by alkaline extraction and acid precipitation. And the structural and functional properties of protein extracts were measured and analyzed. The effect of extraction conditions (liquid/solid ratio 10-20 mL/g), pH 10-12 and 25-45 °C (temperature) on the yield of *H. pluvialis* protein (HP) was carried out using Box-Behnken design. Under optimum extraction conditions: liquid/solid 20 mL/g, pH 11.5 and lower extraction temperature 35 °C, the highest extraction yield (81.36%) of HP was gained, which was close to the predicted value (83.32%). The HP exhibited the better functional properties. The solubility could reach 93.65% at pH 10.0; the foaming capacity and stability were 88.32 and 89.62%, respectively; the emulsifying capacity and stability were 161.52 and 48.2%, respectively; the water holding capacity (WHC) and oil absorption capacity (OAC) were 4.06 and 3.29 g/g, respectively. Fourier Transform infrared spectroscopy (FTIR) data of the HP showed the β -sheet content (30.37%) was the maximum, while α -helix content (14.86%) was the minimum, whereas the content of amino acids was rich in HP. These results demonstrated that the HP had potential use in food industry.

Cultivation of *scenedesmus* sp. using optimized minimal nutrients and flocculants - a potential platform for mass cultivation.

Environ Technol. 2020 Apr;41(10):1284-1297

Authors: Nithiya EM, Fenila F, Vasumathi KK, Premalatha M

Abstract

A major constraint in the microalgal technology is the economics involved in cultivation and harvesting. This work is focussed on the optimization of nutrients for cultivation and harvesting using '*Scenedesmus* sp'. Response surface methodology (RSM) using 'Face centered central composite design' (FCCD) available in Design expert 10.0.4 was used to develop the regression model for optimization of nutrients and flocculation conditions. The optimum nutrient conditions were 500 ppm of urea, 250 ppm of potassium dihydrogen phosphate and 1000 ppm of potassium hydrogen carbonate under artificial light conditions and 500 ppm of urea and 2000 ppm of potassium hydrogen carbonate under sunlight conditions. The optimum conditions were



predicted using the model and compared with experimental data. The model has an R2 value of 0.9769 and 0.9798 for artificial light and sunlight conditions, respectively. In the case of harvesting studies, 98% flocculation efficiency was obtained for a combination of pH 10.4, temperature 45°C, 200 mg/l of leaf powder of *Cassia auriculata*. The model has an R2 value of 0.9989. The present studies indicated that cultivation of *Scenedesmus* sp. with the optimized nutrients and harvesting conditions facilitate a platform for energy efficient mass cultivation.

Regulation of biohydrogen production by protonophores in novel green microalgae *Parachlorella kessleri*.

J Photochem Photobiol B. 2019 Oct;199:111597

Authors: Manoyan J, Gabrielyan L, Kozel N, Trchounian A

Abstract

The green microalgae *Parachlorella kessleri* RA-002 isolated in Armenia can produce biohydrogen (H₂) during oxygenic photosynthesis. Addition of protonophores, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNF) enhances H₂ yield in *P. kessleri*. The maximal H₂ yield of ~2.20 and 2.08 mmol L⁻¹ was obtained in the presence of 15 μM CCCP and 50 μM DNF, respectively. During dark conditions H₂ production by *P. kessleri* was not observed even in the presence of protonophores, indicating that H₂ formation in these algae was mediated by light conditions. The enhancing effect of protonophores can be coupled with dissipation of proton motive force across thylakoid membrane in *P. kessleri*, facilitating the availability of protons and electrons to [Fe-Fe]-hydrogenase, which led to formation of H₂. At the same time H₂ production was not observed in the presence of diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a specific inhibitor of PS II. Moreover, diuron inhibits H₂ yield in *P. kessleri* in the presence of protonophores. The inhibitory effect of diuron coupled with suppression of electron transfer from PS II. The results showed that in these algae operates PS II-dependent pathway of H₂ generation. This study is important for understanding of the mechanisms of H₂ production by green microalgae *P. kessleri* and developing of its biotechnology.

Ecotoxicological and biochemical effects of environmental concentrations of the plastic-bond pollutant dibutyl phthalate on *Scenedesmus* sp.

Aquat Toxicol. 2019 Oct;215:105281

Authors: Cunha C, Paulo J, Faria M, Kaufmann M, Cordeiro N

Abstract

Phthalate esters are highly present in aquatic plastic litter, which can interfere with the biological processes in the wildlife. In this work, the commonly found freshwater microalga



Scenedesmus sp. was exposed to environmental concentrations (0.02, 1 and 100 $\mu\text{g L}^{-1}$) and to a higher concentration (500 $\mu\text{g L}^{-1}$) of dibutyl phthalate (DBP), which is an environmental pollutant. The growth, pH variation, production of photosynthetic pigments, proteins and carbohydrates were evaluated. The main inhibition effect of DBP on the microalgal growth was observed in the first 48 h of the exposure (EC50: 41.88 $\mu\text{g L}^{-1}$). A reduction in the photosynthetic pigment concentration was observed for the 0.02, 1 and 100 $\mu\text{g L}^{-1}$ conditions indicating that the DBP downregulated the growth rate and affected the photosynthetic process. A significant increase in protein production was only observed under 500 $\mu\text{g L}^{-1}$ DBP exposure. The extracellular carbohydrates production slightly decreased with the presence of DBP, with a stronger decrease occurring in the 500 $\mu\text{g L}^{-1}$ condition. These results highlight the environmental risk evaluation and ecotoxicological effects of DBP on the production of biovaluable compounds by microalgae. The results also emphasize the importance of assessing the consequences of the environmental concentrations exposure as a result of the DBP dose-dependent correlation effects.

Comparison of algal harvest and hydrogen peroxide treatment in mitigating cyanobacterial blooms via an in situ mesocosm experiment.

Sci Total Environ. 2019 Dec 01;694:133721

Authors: Fan F, Shi X, Zhang M, Liu C, Chen K

Abstract

The use of short-term, fast-acting curative treatments to rapidly suppress the proliferation of upcoming cyanobacterial blooms without negative side effects on overall water quality is important for environmental regulatory agencies. A 15-day in situ mesocosm experiment was conducted to evaluate the effects of algal harvest at different intensities and the effect of hydrogen peroxide on the mitigation of cyanobacterial blooms, subsequent algal growth and phytoplankton community structure. The results indicate that filtration through a 30- μm -pore-size net could remove most of the *Microcystis* colonies, leading to a decline in algal biomass. However, algal harvest at 30% and 60% intensities tended to promote cyanobacterial growth under nutrient-replete conditions, and the mitigation effect only lasted a few days, since cyanobacteria biomass exhibited no significant difference between the control and those two treatments on Day 6. When the algal harvest intensity was 90%, the cyanobacterial biomass remained at a relatively low level for 15 days. The average *Microcystis* colony size rapidly returned to the initial level after an initial decline across all the algal harvest intensities, indicating that algal harvest should be repeatedly performed within a short time period to mitigate *Microcystis* blooms. Furthermore, removing *Microcystis* colonies by filtration led to increased diversity in the phytoplankton community, as the proportion of non-*Microcystis* cyanobacteria increased with harvest intensity. This result might pose a challenge for cyanobacterial bloom control over the long term if filamentous cyanobacteria become dominant. The 10.0 mg L⁻¹ H₂O₂ treatment selectively suppressed cyanobacteria throughout the experimental period, leading to succession from a cyanobacteria-dominated to a Chlorophyta-dominated community after Day 9. Overall, using hydrogen peroxide is more effective than algal harvesting as a one-time quick curative measure.



Nannochloropsis Extract-Mediated Synthesis of Biogenic Silver Nanoparticles, Characterization and In Vitro Assessment of Antimicrobial, Antioxidant and Cytotoxic Activities.

Asian Pac J Cancer Prev. 2019 08 01;20(8):2353-2364

Authors: Gnanakani PE, Santhanam P, Premkumar K, Eswar Kumar K, Dhanaraju MD

Abstract

Objective: To investigate the biogenic synthesis of silver nanoparticles (AgNPs) using partially purified ethyl acetate extract of *Nannochloropsis* sp. hexane (EAENH) fraction of microalga. **Methods:** The green synthesis of AgNPs was confirmed with UV-Vis spectrum which shows the surface plasmon resonance (SPR) at 421 nm. Fourier Transform Infrared Spectra (FTIR) presented the involvement of functional groups like carboxyl groups of fatty acids, tetraterpenoids of xanthophylls, hydroxyl groups of polyphenols, carbonyl and amide linkage of proteins in the AgNP synthesis. Gas Chromatography-Mass Spectrometry analysis (GCMS) revealed that phytochemicals like octadecanoic acid and hexadecanoic acid imply in capping, bioreduction, and stabilization of AgNPs. **Result:** High-resolution Transmission electron microscope (HRTEM), Dynamic light scattering (DLS), X-ray diffraction (XRD) and EDX analysis showed the crystalline form of the AgNPs with Z-average size 57.25 nm. The zeta potential value of -25.7 mV demonstrated the negative surface charge and colloidal stability of AgNPs. The antimicrobial activity of AgNPs displayed effective inhibition zone against selected bacterial and fungal pathogens. In vitro, antioxidant effects were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide and reducing power assays which revealed excellent scavenging potential for AgNPs than the extracts. The anti-proliferative potential of biofabricated AgNPs and extracts on Human Non-small lung cancer cell line (A549) was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay with IC50 values of 15 µg/mL and 175 µg/mL respectively. **Conclusion:** The study reveals that the microalgae-mediated AgNPs possesses potent antimicrobial and antioxidant activity along with the ability to stimulate apoptosis in A-549 cell line.

Immobilization of mercury using high-phosphate culture-modified microalgae.

Environ Pollut. 2019 Nov;254(Pt A):112966

Authors: Huang R, Huo G, Song S, Li Y, Xia L, Gaillard JF

Abstract

This study developed a novel Hg(II) immobilization strategy by firstly incubating algal cells in high-phosphate cultures for surface modification, followed by obtaining the P-rich biomass as adsorbents for enhanced Hg(II) removal and then charring the Hg-loaded biomass to prevent leaching of phosphate and to immobilize Hg(II). For algal surface modification, *Scenedesmus*



obtusus XJ-15 were cultivated under different P concentrations and obtained the highest sites concentration of surface phosphoryl functional groups in 80 mgL⁻¹ P cultures. For Hg(II) adsorption, biomass from 80 mgL⁻¹ P cultures (B-80) achieved the highest saturated sorption capacity of 95 mg g⁻¹ fitting to Langmuir isotherm model under the optimum pH of 5.0. For charring stabilization, the Hg-loaded B-80 was calcinated under different temperatures, and the product obtained from 300 °C charring showed the lowest Hg(II) leaching rate without P release. Moreover, FT-IR and XPS analysis indicate that the surge of surface phosphoryl functional groups dominated the enhancement of Hg(II) sorption and also Hg(II) charring immobilization. The above results suggested that the developed strategy is promising for both phosphate and mercury removal from water and for co-immobilization of P and Hg(II) to prevent leaching.

pH effects on the lipid and fatty acids accumulation in *Chlamydomonas reinhardtii*.

Biotechnol Prog. 2019 11;35(6):e2891

Authors: Ochoa-Alfaro AE, Gaytán-Luna DE, González-Ortega O, Zavala-Arias KG, Paz-Maldonado LMT, Rocha-Urbe A, Soria-Guerra RE

Abstract

pH variations influence the delivery of essential nutrients and CO₂ solubility, which impact algae metabolism. In this study the microalgal growth and chlorophyll, lipid, and fatty acids content; along with the expression of some genes implicated in the biosynthesis of lipids were examined in *Chlamydomonas reinhardtii* subjected to pH values of 7.0, 7.8, and 8.5. At pH 7.8 an increase in cell growth was observed with a significant accumulation of chlorophyll (1.75-fold) when compared with growth at pH 7, while at pH 8.5 a sharp decrease in both parameters was observed when compared with the other pH values tested. Lipid content increased 3.0 (14.81% of dry cell weight, dcw) and 2.3 times (11.43% dcw) at pH 7.8 and 8.5, respectively, when compared with the experiment at pH 7 (4.97% dcw). The compositions of major fatty acids in the strains growing at pH 7.0, 7.8, or 8.5 were 25.7, 28.0, and 32.1% for palmitic acid; 17.3, 14.7, and 25.7% for oleic acid; and 9.8, 12.1, and 4.6% for linoleic acid; respectively. qRT-PCR analysis showed that the transcripts of β -carboxyltransferase, Acyl carrier protein 1, acyl-ACP thiolase 1, acyl-sn-glycerol-3-phosphate acyltransferase, and diacylglycerol acyl transferase isoform 3 were significantly induced at pH 7.8 when compared with the other two pH conditions. These results indicate that the induction of genes implicated in the early and final steps of lipid biosynthesis contributes to their accumulation in the stationary phase. Our research suggests that a pH of 7.8 might be ideal to maximize growth and lipid accumulation.

Optimization, isolation, characterization and hepatoprotective effect of a novel pigment-protein complex (phycocyanin) producing microalga: *Phormidium versicolor* NCC-466 using response surface methodology.

Int J Biol Macromol. 2019 Sep 15;137:647-656



Abstract

In our study, we focused on the optimization; antioxidant and hepatoprotective potentials of novel pigment-protein complex(C-PC) isolated from *Phormidium versicolor* against cadmium induced liver injury in rats. From analysis, the C-PC extraction parameters were optimized using the response surface methodology (RSM) for optimal recoveries of C-PC extraction. For analysis, the optimum operational conditions for maximizing phycocyanins concentration (67.45mg/g DM) were found to be water/solid 2, temperature 32.5°C and pH7.2. This pigment was identified using HPLC and FTIR analysis. In addition, the molecular masses of α and β subunits were 17 and 19kDa. Scavenging activity of superoxide anion, hydroxyl, nitric oxide radicals and metal chelating in vitro results indicated that C-PC has an excellent capacity as antioxidant. In vivo study, C-PC significantly prevented cadmium-induced elevation of ALAT, ASAT and bilirubin levels in rats. The histopathological observations supported the results serum enzymes assays. The results of this study revealed that C-PC has significant hepatoprotective potential. C-PC (50mgkg⁻¹ body weight) significantly enhanced the levels of antioxidant enzymes. It can be concluded that C-PC possesses prevention action against hepatotoxicity caused by cadmium.

Graphene-based materials do not impair physiology, gene expression and growth dynamics of the aeroterrestrial microalga *Trebouxia gelatinosa*.

Nanotoxicology. 2019 05;13(4):492-509

Authors: Banchi E, Candotto Carniel F, Montagner A, Bosi S, Bramini M, Crosera M, León V, Martín C, Pallavicini A, Vázquez E, Prato M, Tretiach M

Abstract

The effects of two graphene-based materials (GBMs), few-layers graphene (FLG) and graphene oxide (GO), were studied in the aeroterrestrial green microalga *Trebouxia gelatinosa*. Algae were subjected to short- and long-term exposure to GBMs at 0.01, 1 and 50 $\mu\text{g mL}^{-1}$. GBMs internalization after short-term exposures was investigated with confocal microscopy, Raman spectroscopy and TEM. Potential negative effects of GBMs, compared to the oxidative stress induced by H₂O₂, were verified by analyzing chlorophyll a fluorescence (ChlaF), expression of stress-related genes and membrane integrity. Effects of up to 4-week-long exposures were assessed analyzing growth dynamics, ChlaF and photosynthetic pigments. GBMs were not observed in cells but FLG was detected at the interface between the cell wall and plasma membrane, whereas GO was observed adherent to the external wall surface. FLG caused the down-regulation of the HSP70-1 gene, with the protein levels remaining stable, whereas GO had no effect. In comparison, H₂O₂ produced dose- and time-dependent effects on ChlaF, gene expression and HSP70 protein level. Long-term exposures to GBMs did not affect growth dynamics, ChlaF or photosynthetic pigment contents, indicating that the few observed short-term effects were not dangerous on the long-term. Results suggest that interactions between FLG and plasma membrane were harmless, activating a down-regulation of the HSP70-1 gene similar to that induced by H₂O₂. Our work shows that studying GBMs effects on non-model



organisms is important since the results of model green microalgae are not representative of the whole taxonomic group.

Impact of Culture Conditions on Neutral Lipid Production by Oleaginous Yeast.

Methods Mol Biol. 2019;1995:311-325

Authors: Fakankun I, Mirzaei M, Levin DB

Abstract

Oleaginous yeasts have the ability to accumulate and store triacylglycerides (TAGs) to more than 20% of their cell mass. Oleaginous yeasts have advantages over oil seed plants and microalgae because they grow much faster (doubling time is usually less than an hour), accumulate cell mass to much higher densities, and are less affected by seasonal or weather conditions. The TAGs synthesized by oleaginous yeasts are often rich in polyunsaturated fatty acids and can be used either for biodiesel production or as edible oils. "Red" yeasts are oleaginous yeasts that can synthesize and accumulate high concentrations of TAGs. Many factors affect the growth of red yeasts and subsequent yields of TAGs. These factors include carbon and nitrogen sources, their concentrations, the C/N ratio, temperature, pH, aeration rate, mineral elements, inorganic salts, and inhibitors. The effect of each factor varies with the yeast strain and its growth phase. *Rhodospiridium diobovatum* is a "red" yeast that can utilize low-cost substrates, such as waste glycerol derived from biodiesel production as a carbon source, and can synthesize and accumulate high concentrations of both TAGs and carotenoids.

Optimization of pH induced flocculation of marine and freshwater microalgae via central composite design.

Biotechnol Prog. 2019 05;35(3):e2801

Authors: Akış S, Özçimen D

Abstract

Microalgae harvesting via pH induced flocculation along with utilization of recovered medium after flocculation is one of the most economical methods for separating the microalgal biomass in order to reduce the dewatering cost. In this study, optimization of marine and freshwater microalgae flocculation by pH adjustment was investigated via central composite design methodology. One molar of KOH and NaOH solutions were used to increase the pH level of the microalgal culture. Increasing pH value of the medium provided the highest flocculation efficiency up to 92.63 and 86.18% with pH adjusted to 10.5 with KOH and NaOH solutions for marine microalgae *Nannochloropsis oculata* and freshwater microalgae *Chlorella minutissima*, respectively. Also, it was revealed that microalgae cells were still alive after flocculation process and their biochemical composition was not changed, and flocculated medium can be used again



for the next microalgal production. According to the results, it can be said that this method is cheap and effective, simple to operate and provides the utilization of flocculated medium again.

Eco-friendly rapid removal of triclosan from seawater using biomass of a microalgal species: Kinetic and equilibrium studies.

J Hazard Mater. 2019 05 05;369:674-683

Authors: Santaefemia S, Abalde J, Torres E

Abstract

Triclosan is an important emerging pollutant. It has become ubiquitous due to its incomplete elimination in municipal wastewater treatment plants causing serious environmental problems. Biomass from microorganisms as sorbent of pollutants can be an eco-friendly alternative for triclosan removal. In this work, the elimination of triclosan using biomass (dead and living) of the marine microalga *Phaeodactylum tricornutum* was characterized in cultures exposed to light and in a complex solution (seawater). Maximum removal capacity, isotherms, kinetics, FTIR characterization, pH effect and reuse were evaluated and discussed. Photodegradation of triclosan was also evaluated. Both biomasses showed similar effectiveness; around 100% of pollutant was eliminated when its concentration was 1 mg L⁻¹ in only 3 h using a biomass concentration of 0.4 g L⁻¹. A pseudo-second order model guided the biosorption process. Considering the photodegradation as a first-order process, the whole process (photodegradation + biosorption) was suitably modelled with pseudo-third order and Elovich kinetics. Biosorption increased with the decrease in pH. Temkin isotherm showed the best fit for the experimental data. Both biomasses showed good reuse after five cycles, losing only 7% in efficiency. *P. tricornutum* biomass is an attractive eco-material for triclosan elimination with low-cost and easy handling than other sorbents.

Solubility and aggregation behavior of protein fractions from the heterotrophically cultivated microalga *Chlorella protothecoides*.

Food Res Int. 2019 02;116:283-290

Authors: Grossmann L, Hinrichs J, Weiss J

Abstract

Protein solubility in water is a key property of food proteins. The aim of this work was to study the solubility and microstructural properties as a function of pH of both protein fractions (water-soluble (WSPE) and water-insoluble protein extracts (WISPE)) obtained from the microalga *Chlorella protothecoides*, which is promising for food use. Protein solubility was determined as the ratio of protein concentration in the supernatant after centrifugation to total protein concentration. An unusually high solubility and only slight gravitational separation across a very broad pH-range (2-12) were observed for the WSPE with a minimum protein solubility of



84.3 ± 2.2% at pH 2. The origin of this high pH-independent protein solubility was attributed to a high degree of glycosylation and a high amount of hydrophilic amino acids. In contrast, the WISPE was found to contain strongly aggregated proteins, and these large aggregates separated rapidly from solution by gravitation independent of the pH. This corresponded to their protein solubility, which was overall low in the pH-range of 2-11, and only increased at pH 12 to a maximum solubility of 26.9 ± 2.8%. These results suggest that the WSPE of *Chlorella protothecoides* may exhibit unique properties for food formulations, allowing for example for both acidified, neutral or slightly alkaline foods to be formulated, whereas WISPE may be more suited for foods where phase separation is rather slow.

A water-soluble [60]fullerene-derivative stimulates chlorophyll accumulation and has no toxic effect on *Chlamydomonas reinhardtii*.

Acta Biochim Pol. 2019 Jul 07;66(3):257-262

Authors: Lang J, Melnykova M, Catania M, Inglot A, Zyss A, Mikruta K, Firgolska D, Wieremiejczuk A, Książek I, Serda M, Nalepa P, Pluciński B, Giza A, Jedynak P

Abstract

Chlamydomonas reinhardtii (WT 2137) P. A. Dang. (Volvocales, Chlorophyceae) is a green microalgae serving as a suitable model in scientific research and a promising industrial biotechnology platform for production of biofuel, hydrogen and recombinant proteins. Fullerenes (C60) are allotropic carbon nanoparticles discovered in 1985 and used in biomedical studies since the early 1990s, when water solubilization methodologies were developed. Recently, surface-modified hydroxylated derivatives of fullerenes were proven to enhance algal growth and drought tolerance in plants. Here, a novel type of water-soluble [60]fullerene derivative with 12 glycine residues (GF) has been synthesized and tested for acute toxicity (up to 50 µg/ml) and as a potential biostimulant of algal growth. The effects of GF on pigment composition and growth rate of *Chlamydomonas reinhardtii* were systematically investigated. Our results suggest that GF was not toxic, and no negative change in the pigment content and no stress symptoms were observed. No changes in the photosynthetic parameters based on the fluorescence of chlorophyll a in Photosystem II (NPQ, Fv/Fm, Fv/F0, PI and RC/ABS) were observed. The GF had no effect on cell size and growth rate. At a concentration of 20 µg/ml, GF stimulated chlorophyll accumulation in 3-day-old cultures.

The effect of pH on the acute toxicity of phenanthrene in a marine microalgae *Chlorella salina*.

Sci Rep. 2018 12 04;8(1):17577

Authors: Chen H, Zhang Z, Tian F, Zhang L, Li Y, Cai W, Jia X



Abstract

Phenanthrene is one of the most abundant polycyclic aromatic hydrocarbons (PAHs) found in continental shelf environment of China and is on the EPA's Priority Pollutant list. In this study, the effects of phenanthrene on marine algal growth rate were determined after 96-h exposure at pH 6.0, 7.0, 8.0, 9.0, and 10.0 in seawater of salinity 35. Two measuring techniques to assess growth inhibition were also compared using prompt fluorescence and microscopic cell count. The results showed that the toxicity of phenanthrene increased significantly ($p < 0.05$) with decreasing pH, with the nominal concentration required to inhibit growth rate by 50%, EC50, decreasing from 1.893 to 0.237 mg L⁻¹ as pH decreased from 9.0 to 6.0, with a decrease higher than 55% from 10.0 to 9.0. In addition, the nominal EC50 values calculated in this study were at the same range of some environmental concentrations of phenanthrene close to areas of crude oil exploration. Based on the two measuring techniques, the results showed that cell count and fluorescence measurement were significantly different ($p < 0.05$), and the nominal EC50 values calculated with cell count measurement were significantly higher than fluorescence measurement at pH 8.0, 9.0 and 10.0. In conclusion, the present studies confirmed that acidification of seawater could affect the toxicity of phenanthrene to this species of microalgae, and which encouraged further studies involving responses of marine organisms to ocean acidification.

Growth and photosynthetic activity of *Chlamydomonas reinhardtii* entrapped in lens-shaped silica hydrogels.

J Biotechnol. 2019 Aug 20;302:58-66

Authors: Homburg SV, Kruse O, Patel AV

Abstract

Entrapment of microalgae in silica hydrogels enables the application as biocatalysts in continuous production of secreted products. Despite a mitigation of substrate and product diffusion limitations by lens-shaped particles, there are no reports on light supply and limitation. This study investigated the impact of hydrogel structure, particle size and biomass loading on the behaviour of the microalga *Chlamydomonas reinhardtii* entrapped in lens-shaped silica particles. Entrapment in tetraethyl orthosilicate and tetra(n-propylamino)silane based hydrogels reduced the growth rate by 30% and 23%, respectively. In contrast, cells entrapped in sodium silicate based hydrogels displayed a growth rate similar to free cells and cells entrapped in calcium alginate (1.13 d⁻¹), indicating a suitable biocompatibility. Reduction of lens height by 26% maintained the growth rate in silica hydrogel. A fourfold increase in biomass loading reduced the growth rate by 20% and elevated the yield coefficient by 211%, indicating the impact of biomass loading on light and nutrient supply on photosynthetic growth. Finally, hydrogen production was observed by entrapped cells. The results of this work will pave the way for robust biocatalytic processes where photosynthetically active cells are protected against harmful mechanical and biological influences.



EVENTOS

AlgaeEurope 2020 - 01 al 03 diciembre 2020

<https://algaeurope.org/>

(Deadline 27 septiembre abstracts)

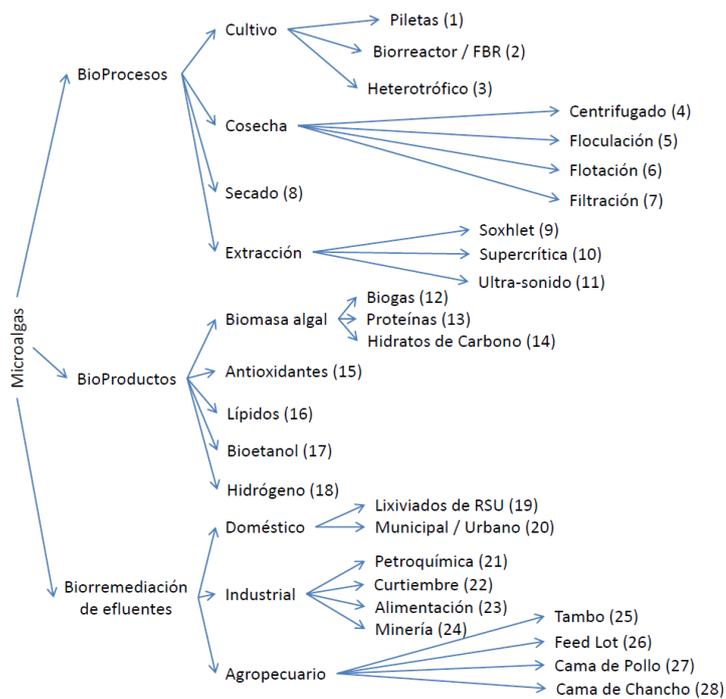
IPC 2021 - International Phycological Congress - 22 al 26 marzo 2021

<https://ipc2021.com/>

(Deadline Registration and Abstract Submission 01 diciembre 2020)

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