# UNIDAD DE VIGILANCIA TECNOLÓGICA E INTELIGENCIA COMPETITIVA

# Microalgas

Mayo de 2022

Ministerio de Agricultura, Ganadería y Pesca
Argentina

# Contenido

## PUBLICACIONES

### A Comparison of β-Carotene, Phytoene and Amino Acids Production in Dunaliella salina DF 15 (CCAP 19/41) and Dunaliella salina CCAP 19/30 Using Different Light Wavelengths

Autor: Yixing Sui

Foods. 2021 Nov 16;10(11):2824. doi: 10.3390/foods10112824.

ABSTRACT

Strains of Dunaliella salina microalgae are of considerable research and industrial interest because they hyper-accumulate β-carotene as well as produce high-quality protein. To explore the co-production of valuable compounds in D. salina, this study compared the production of β-carotene, phytoene and amino acids in two strains cultivated under white, red or blue light until no further nitrogen was available. D. salina DF15 (CCAP 19/41 (PLY DF15)) produced more than 12% β-carotene (ash-free dry weight (AFDW) basis), and red light triggered the production of 9-cis β-carotene at a 9-cis/all-trans β-carotene ratio of 1.5. Phytoene production was also evident in D. salina DF15 under all conditions, particularly under blue light. However, the profile of essential amino acids (EAAs) and calculation of the essential amino acid index (EAAI) was less than ideal in terms of protein quality, for both strains. Umami compounds, quantified as monosodium glutamate (MSG) equivalents, indicated a higher equivalent umami concentration (EUC) in D. salina DF15 under red light (3.2 g MSG/100 g AFDW) than in D. salina CCAP19/30. Overall, D. salina DF15 demonstrates valuable traits for further exploration and product optimisation.

### A Fast-Growing Oleaginous Strain of Coelastrella Capable of Astaxanthin and Canthaxanthin Accumulation in Phototrophy and Heterotrophy

Autor: Amélie Corato

Life (Basel). 2022 Feb 23;12(3):334. doi: 10.3390/life12030334.

ABSTRACT

Considering the importance of microalgae as a promising feedstock for the production of both low- and high-value products, such as lipids and pigments, it is desirable to isolate strains which simultaneously accumulate these two types of products and grow in various conditions in order to widen their biotechnological applicability. A novel freshwater strain from the genus Coelastrella was isolated in Belgium. Compared to other Coelastrella species, the isolate presented rapid growth in phototrophy, dividing 3.5 times per day at a light intensity of 400 µmol·m-2·s-1 and 5% CO2. In addition, nitrogen depletion was associated with the accumulation of astaxanthin, canthaxanthin, and fatty acids, which reached ~30% of dry weight, and a majority of SFAs and MUFAs, which are good precursors for biodiesel. This strain also accumulated astaxanthin and canthaxanthin in heterotrophy. Although the content was very low in this latter condition, it is an interesting feature considering the biotechnological potential of the microalgal heterotrophic growth. Thus, due to its rapid growth in the light, its carotenogenesis, and its fatty acids characteristics, the newly identified Coelastrella strain could be considered as a potential candidate for biorefinery purposes of both low- and high-values products.

## A novel Penicillium sumatraense isolate reveals an arsenal of degrading enzymes exploitable in algal bio-refinery processes

Autor: M Giovannoni

Biotechnol Biofuels. 2021 Sep 13;14(1):180. doi: 10.1186/s13068-021-02030-9.

ABSTRACT

BACKGROUND: Microalgae are coming to the spotlight due to their potential applications in a wide number of fields ranging from the biofuel to the pharmaceutical sector. However, several factors such as low productivity, expensive harvesting procedures and difficult metabolite extractability limit their full utilization at industrial scale. Similarly to the successful employment of enzymatic arsenals from lignocellulolytic fungi to convert lignocellulose into fermentable sugars for bioethanol production, specific algalytic formulations could be used to improve the extractability of lipids from microalgae to produce biodiesel. Currently, the research areas related to algivorous organisms, algal saprophytes and the enzymes responsible for the hydrolysis of algal cell wall are still little explored.

RESULTS: Here, an algal trap method for capturing actively growing microorganisms was successfully used to isolate a filamentous fungus, that was identified by whole-genome sequencing, assembly and annotation as a novel Penicillium sumatraense isolate. The fungus, classified as P. sumatraense AQ67100, was able to assimilate heat-killed Chlorella vulgaris cells by an enzymatic arsenal composed of proteases such as dipeptidyl- and amino-peptidases, β-1,3-glucanases and glycosidases including α- and β-glucosidases, β-glucuronidase, α-mannosidases and β-galactosidases. The treatment of C. vulgaris with the filtrate from P. sumatraense AQ67100 increased the release of chlorophylls and lipids from the algal cells by 42.6 and 48.9%, respectively.

CONCLUSIONS: The improved lipid extractability from C. vulgaris biomass treated with the fungal filtrate highlighted the potential of algal saprophytes in the bioprocessing of microalgae, posing the basis for the sustainable transformation of algal metabolites into biofuel-related compounds.

## Acid Tolerant and Acidophilic Microalgae: An Underexplored World of Biotechnological Opportunities

Autor: Fabian Abiusi

Front Microbiol. 2022 Jan 27;13:820907. doi: 10.3389/fmicb.2022.820907. eCollection 2022.

ABSTRACT

Despite their large number and diversity, microalgae from only four genera are currently cultivated at large-scale. Three of those share common characteristics: they are cultivated mainly autotrophically and are extremophiles or tolerate "extreme conditions." Extreme growth conditions aid in preventing contamination and predation of microalgae, therefore facilitating outdoor cultivation. In search for new extremophilic algae suitable for large-scale production, we investigated six microalgal strains able to grow at pH below 3 and belonging to four genera; Stichococcus bacillaris ACUF158, Chlamydomonas acidophila SAG 2045, and Chlamydomonas pitschmannii ACUF238, Viridiella fridericiana ACUF035 and Galdieria sulphuraria ACUF064 and ACUF074. All strains were cultivated autotrophically at light intensity of 100 and 300 μmol m-2 s-1 and pH between 1.9 and 2.9. The autotrophic biomass productivities were compared with one of the most productive microalgae, Chlorella sorokiniana SAG 211-8K, grown at pH 6.8. The acid tolerant strains have their autotrophic biomass productivities reported for the first time. Mixotrophic and heterotrophic properties were investigated when possible. Five of the tested strains

displayed autotrophic biomass productivities 10-39% lower than Chlorella sorokiniana but comparable with other commercially relevant neutrophilic microalgae, indicating the potential of these microalgae for autotrophic biomass production under acidic growth conditions. Two acid tolerant species, S. bacillaris and C. acidophila were able to grow mixotrophically with glucose. Chlamydomonas acidophila and the two Galdieria strains were also cultivated heterotrophically with glucose at various temperatures. Chlamydomonas acidophila failed to grow at 37°C, while G. sulphuraria ACUF64 showed a temperature optimum of 37°C and G. sulphuraria ACUF74 of 42°C. For each strain, the biomass yield on glucose decreased when cultivated above their optimal temperature. The possible biotechnological applications of our findings will be addressed.

## Aerobes and phototrophs as microbial organic fertilizers: Exploring mineralization, fertilization and plant protection features

Autor: Eva Wambacq

PLoS One. 2022 Feb 2;17(2):e0262497. doi: 10.1371/journal.pone.0262497. eCollection 2022.

ABSTRACT

Organic fertilizers and especially microbial biomass, also known as microbial fertilizer, can enable a paradigm shift to the conventional fertilizer-to-food chain, particularly when produced on secondary resources. Microbial fertilizers are already common practice (e.g. Bloom® and Synagro); yet microbial fertilizer blends to align the nutrient release profile to the plant's needs are, thus far, unexplored. Moreover, most research only focuses on direct fertilization effects without considering added value properties, such as disease prevention. This study has explored three promising types of microbial fertilizers, namely dried biomass from a consortium of aerobic heterotrophic bacteria, a microalga (Arthrospira platensis) and a purple non-sulfur bacterium (Rhodobacter sphaeroides). Mineralization and nitrification experiments showed that the nitrogen mineralization profile can be tuned to the plant's needs by blending microbial fertilizers, without having toxic ammonium peaks. In a pot trial with perennial ryegrass (Lolium perenne L.), the performance of microbial fertilizers was similar to the reference organic fertilizer, with cumulative dry matter yields of 5.6-6.7 g per pot. This was confirmed in a pot trial with tomato (Solanum lycopersicum L.), showing an average total plant length of 90-99 cm after a growing period of 62 days for the reference organic fertilizer and the microbial fertilizers. Moreover, tomato plants artificially infected with powdery mildew (Oidium neolycopersici), a devastating disease for the horticultural industry, showed reduced disease symptoms when A. platensis was present in the growing medium. These findings strengthen the application potential of this novel class of organic fertilizers in the bioeconomy, with a promising match between nutrient mineralization and plant requirements as well as added value in crop protection.

## Alternative protein sources of plant, algal, fungal and insect origins for dietary diversification in search of nutrition and health

Autor: Marília Aparecida Fidelis E Moura

Crit Rev Food Sci Nutr. 2022 Jun 14:1-18. doi: 10.1080/10408398.2022.2085657. Online ahead of print.

ABSTRACT

This review aimed to compare alternative protein sources in terms of nutritional composition and health benefits with the purpose of disseminating up-to-date knowledge and contribute for diversification of the food marked and consumers decision-making. Plant-based is the most well-established category of alternative proteins, but there is still room for diversification. Less conventional species such as chia seeds are prominent sources of ω-3 (~60% total lipids), while hempseed and quinoa are notable sources of ω-6

(up to 58% and 61%, respectively). Edible insects and microalgae are alternative foods rich in protein (up to 70%), fibers (~30%), as well as peptides and polysaccharides with antimicrobial, antioxidant, anti-hypertensive, antidiabetic, antidepressant, antitumor, and immunomodulatory activities. Additionally, lipid contents in insect larvae can be as high as 50%, on a dry weight basis, containing fatty acids with anti-inflammatory and antitumor properties. In contrast, edible fungi have low lipid contents (~2%), but are rich in carbohydrates (up to 79%) and have balanced amino acid profiles. The results suggest that food formulations combining different alternative protein sources can meet dietary requirements. Further studies on flavoring and texturing processes will help to create meat and dairy analogs, thus helping to broaden acceptance and applicability of alternative protein sources.

## An automated, modular system for organic waste utilization using heterotrophic alga Galdieria sulphuraria: Design considerations and sustainability

Autor: Maximilian Julius Pahmeyer

Bioresour Technol. 2022 Mar;348:126800. doi: 10.1016/j.biortech.2022.126800. Epub 2022 Feb 1.

ABSTRACT

Large amounts of food are wasted and valuable contents are not utilized completely. Methods to process such wastes into biomass of defined composition automatically and in decentralized locations are lacking. Thus, this study presents a modular design for residue utilization and continuous production of the heterotrophic alga Galdieria sulphuraria. A life cycle and economic assessment are carried out on the hypothetical design to define whether the proposed system can be ecologically and economically viable. Producing one kg of dried biomass would cost 4.38 € and be associated with 3.8 kg CO2 eq emitted, 69.9 MJ of non-renewable energy use, and 0.09 m2 of land occupation. Sustainability is comparable to conventional protein sources, with further improvement foreseen through avoidance of drying. These results demonstrate how circular bioeconomy potentials of residues could be realized using heterotrophic G. sulphuraria. It highlights key issues of developing an environmentally and economically sustainable concept.

## Arthrospira sp. mediated bioremediation of gray water in ceramic membrane based photobioreactor: process optimization by response surface methodology

Autor: Shritama Mukhopadhyay

Int J Phytoremediation. 2022 Jan 25:1-12. doi: 10.1080/15226514.2022.2027865. Online ahead of print.

ABSTRACT

Direct discharge of raw domestic sewage enriched with nitrogenous and phosphorous compounds into the water bodies causes eutrophication and other environmental hazards with detrimental impacts on public and ecosystem health. The present study focuses on phycoremediation of gray water with Arthrospira sp. using an innovative hydrophobic ceramic membrane-based photobioreactor system integrated with CO2 biofixation and biodiesel production, aiming for green technology development. Surfactant and oil-rich gray water collected from the domestic kitchen was used wherein, chloride, sulfate, and surfactant concentrations were statistically optimized using response surface methodology (RSM), considering maximum microalgal growth rate as a response for the design. Ideal concentrations (mg/L) of working parameters were found to be 7.91 (sulfate), 880.49 (chloride), and 144.02 (surfactant), respectively to achieve optimum growth rate of 0.43

gdwt/L/day. Enhancement of growth rate of targeted microalgae by 150% with suitable CO2 (19.5%) supply and illumination in the photobioreactor affirms its efficient operation. Additionally, harvested microalgal biomass obtained from the process showed a biodiesel content of around 5.33% (dry weight). The microalgal treatment enabled about 96.82, 87.5, and 99.8% reductions in BOD, COD, and TOC, respectively, indicating the potential of the process in pollutant assimilation and recycling of such wastewater along with value-added product generation.

## Biofuel recovery from microalgae biomass grown in dairy wastewater treated with activated sludge: The next step in sustainable production

Autor: Henrique Vieira de Mendonça

Sci Total Environ. 2022 Feb 14:153838. doi: 10.1016/j.scitotenv.2022.153838. Online ahead of print.

ABSTRACT

Microalgae biofuel could be the next step in avoiding the excessive use of fossil fuels and reducing negative impacts on the environment. In the present study, two species of microalgae (Scenedesmus obliquus and Chlorella vulgaris) were used for biomass production, grown in dairy wastewater treated by activated sludge systems. The photobioreactors were operated in batch and in continuous mode. The dry biomass produced was in the range of 2.30 to 3.10 g L-1. The highest volumetric yields for lipids and carbohydrates were 0.068 and 0.114 g L-1 day-1. Maximum CO2 biofixation (750 mg L-1 day-1) was obtained in continuous mode. The maximum values for lipids (21%) and carbohydrates (39%) were recorded in the batch process with species Scenedesmus obliquus. In all of the experiments, the Linolenic acid concentration (C18:3) was greater than 12%, achieving satisfactory oxidative stability and good quality. Projected biofuel production could vary between 4,863,708 kg and 9,246,456 kg year-1 if all the dairy wastewater produced in Brazil were used for this purpose. Two hectares would be needed to produce 24,99 × 109 L year-1 of microalgae bioethanol, a far lower value than used in cultivating sugar cane. If all dairy wastewater generated annually in Brazil were used to produce microalgae biomass, it would be possible to obtain approximately 30,609 to 53,647 barrels of biodiesel per year. These data show that only by using dairy wastewater would biofuels be produced to replace 17% to 40% of the fossil fuels currently used in Brazil.

## Biopolymers production from microalgae and cyanobacteria cultivated in wastewater: Recent advances

Autor: Savvas Giannis Mastropetros

Biotechnol Adv. 2022 Jun 3;60:107999. doi: 10.1016/j.biotechadv.2022.107999. Online ahead of print.

ABSTRACT

Plastic materials are used to manufacture a broad variety of items with a short useful lifespan, resulting in significant amounts of waste material generation. This form of waste is often observed floating at sea, and different microplastics have been discovered in fish stomachs and women's placentas. Bioplastics are a more biodegradable substitute for fossil-based polymers. Microalgae are capable of producing poly (hydroxy alkanoate) esters (PHAs), aliphatic polyesters that are biodegradable. The most prevalent and well-characterized biopolymer is the poly (3-hydroxy butyrate) ester (PHB), which belongs to the short-chain PHAs. Under aerobic conditions, PHB compounds degrade fully to carbon dioxide and water. They are ecologically neutral, having thermal and mechanical qualities comparable to those of petrochemical polymers. Numerous microalgae species have been reported in the literature to be capable of making bioplastics under certain conditions (N-P restriction, light exposure, etc.), which may be exploited as a source of energy and carbon.

To further ameliorate the environmental impact of microalgae culture for bioplastics production, a limited number of published studies have examined the accumulation of bioplastics, from microalgae grown in wastewater, at a concentration of 5.5-65% of dry biomass weight.

## Bioprocess Optimization for the Production of Arthrospira (Spirulina) platensis Biomass Enriched in the Enzyme Alkaline Phosphatase

Autor: Giorgos Markou

Bioengineering (Basel). 2021 Oct 15;8(10):142. doi: 10.3390/bioengineering8100142.

ABSTRACT

The enzyme alkaline phosphatase (ALP) is gaining interest because it exerts bioactive properties and may be a potentially important therapeutic agent for many disorders and diseases. Microalgae are considered an important novel source for the production of diverse bio-compounds and are gaining momentum as functional foods/feeds supplements. So far, studies for the production of ALP are limited to mammalian and partly to some heterotrophic microbial sources after its extraction and/or purification. Methods: Arthrospira was cultivated under P-limitation bioprocess and the effect of the P-limitation degree on the ALP enrichment was studied. The aim of this work was to optimize the cultivation of the edible and generally-recognized-as-safe (GRAS) cyanobacterium Arthrospira platensis for the production of single-cell (SC) biomass enriched in ALP as a potential novel functional diet supplement. Results: The results revealed that the relationship between intracellular-P and single-cell alkaline phosphatase (SC-ALP) activity was inverse; SC-ALP activity was the highest (around 50 U g-1) when intracellular-P was the lowest possible (around 1.7 mg-P g-1) and decreased gradually as P availability increased reaching around 0.5 U g-1 in the control cultures. Under the strongest P-limited conditions, a more than 100-fold increase in SC-ALP activity was obtained; however, protein content of A. platensis decreased significantly (around 22-23% from 58%). Under a moderate P-limitation degree (at intracellular-P of 3.6 mg-P g-1), there was a relatively high SC-ALP activity (>28 U g-1) while simultaneously, a relative high protein content (46%) was attained, which reflects the possibility to produce A. platensis enriched in ALP retaining though its nutritional value as a protein rich biomass source. The paper presents also results on how several parameters of the ALP activity assay, such as pH, temperature etc., and post-harvest treatment (hydrothermal treatment and biomass drying), influence the SC-ALP activity.

## Bioprospecting and selection of tolerant strains and productive analyses of microalgae grown in vinasse

Autor: Camila Candido

Braz J Microbiol. 2022 Feb 9. doi: 10.1007/s42770-022-00692-7. Online ahead of print.

ABSTRACT

In order to contribute to the biotechnology of microalgae cultivated in vinasse, we carried out the bioprospection of tolerant species and synthesized biomolecules of the total biomass (microalgae and bacteria), recovered from cultures. To use vinasse as a culture medium for the microalgae, waste was centrifuged and used in concentrations from 5 to 50%. Daily cell densities, growth rates, and EC50 values were obtained. After defining the best pair of vinasse concentration/microalgae strain, dry biomass, and composition (proteins and carbohydrates) were determined in 96 h cultures, considering the associated community (bacteria and yeast). The microalgae tested were Chlamydomonas sp., Chlorella sorokiniana, Chlorella vulgaris, Desmodesmus spinosus, Haematococcus pluvialis, Monoraphidium sp., Scenedesmus quadricauda, and Tetraselmis gracilis. The results showed that although the microalgal growth rates in vinasse were similar to controls in BG11, the cells in vinasse had higher biovolumes, dry biomass, and total proteins. The

species H. pluvialis, S. quadricauda, and T. gracilis showed the best productivity parameters in vinasse, despite lower growth rates than the other species. Using low concentrations of centrifuged vinasse as a culture medium, only 22% of biological contaminants were present, thus most of the processed biomass was mainly composed of microalgae. Thus, Chlamydomonas sp., D. spinosus, S. quadricauda, and H. pluvialis microalgae have attributes such as resistance and biomolecules that make them candidates for further optimization in production systems, combining the environmental benefits of using waste with the production of biomolecules and/or biomass of commercial interest.

## Biosynthesis of microalgal lipids, proteins, lutein, and carbohydrates using fish farming wastewater and forest biomass under photoautotrophic and heterotrophic cultivation

Autor: Sachin Vyas

Bioresour Technol. 2022 Sep;359:127494. doi: 10.1016/j.biortech.2022.127494. Epub 2022 Jun 17.

ABSTRACT

Biorefineries enable the circular, sustainable, and economic use of waste resources if value-added products can be recovered from all the generated fractions at a large-scale. In the present studies the comparison and assessment for the production of value-added compounds (e.g., proteins, lutein, and lipids) by the microalga Chlorella sorokiniana grown under photoautotrophic or heterotrophic conditions was performed. Photoautotrophic cultivation generated little biomass and lipids, but abundant proteins (416.66 mg/gCDW) and lutein (6.40 mg/gCDW). Heterotrophic conditions using spruce hydrolysate as a carbon source favored biomass (8.71 g/L at C/N 20 and 8.28 g/L at C/N 60) and lipid synthesis (2.79 g/L at C/N 20 and 3.61 g/L at C/N 60) after 72 h of cultivation. Therefore, heterotrophic cultivation of microalgae using spruce hydrolysate instead of glucose offers a suitable biorefinery concept at large-scale for biodiesel-grade lipids production, whereas photoautotrophic bioreactors are recommended for sustainable protein and lutein biosynthesis.

## Buffering culture solution significantly improves astaxanthin production efficiency of mixotrophic Haematococcus pluvialis

Autor: Rongrong Lv

Bioresour Technol. 2022 Jun;354:127175. doi: 10.1016/j.biortech.2022.127175. Epub 2022 Apr 19.

ABSTRACT

Sodium acetate (NaAc) supplementation, often used to increase the growth of H. pluvialis under low light, but promotes cell death under high light; its underlying reasons and solutions are rarely reported. Here, NaAc supplementation was found to rapidly increase pondus hydrogenii (pH) of culture solution, elevate reactive oxygen species (ROS), and cause cell death immediately under higher light. Adjusting pH of NaAc supplemented culture solution with 10 mM Tris-HCl once before high light significantly reduced cell mortality and increased astaxanthin yield. When verified in a 5-litre photobioreactor, this novel method produced over 4.0% of dry weight (DW) astaxanthin within only 8-10 days. In summary, this study explained reasons underlying NaAc supplementation-induced cell death and provided a rapid, easy and effective method to produce high amount of astaxanthin in H. pluvialis.

## CRISPRa/i with Adaptive Single Guide Assisted Regulation DNA (ASGARD) mediated control of Chlorella sorokiniana to enhance lipid and protein production

Autor: Jia-Yi Lin

Biotechnol J. 2021 Nov 20:e2100514. doi: 10.1002/biot.202100514. Online ahead of print.

ABSTRACT

Chlorella species are indispensable microalgae for biorefinery but are hardly in DNA manipulation due to the high guanine-cytosine (GC) contents of DNA. In this study, we established a new approach via 20 guanines for sgRNA design, which is annotated as "Adaptive Single Guide Assisted Regulation DNA (ASGARD)" and coupling with CRISPR interference associated dCas9 system to overcome the difficulties. At first, C. sorokiniana was predominate as its faster growth rate when compared to C. vulgaris and C. variabilis in the culture using Tris-acetate-phosphate (TAP) medium. Among all the genetic transformants, gene regulation via CRISPRa-VP64 (CRISPRa) enhanced the protein contents up to 60% (w/w) of dry cell weight, where the highest concentration was 570 mg L-1 . Meanwhile, CRISPRi-KRAB (CRISPRi) with ASGARD increased protein content to 65% and lipid formed in the range of 150-250 mg L-1 . From the transcriptome analysis, we deciphered 468 genes down-regulated and 313 genes up-regulated via CRISPRi, while less difference existed in CRISPRa. This novel design and technology reveal a high potential of gene-regulating approach to other species for the biorefinery and bio-industry.

## Ca(2+) participates in the regulation of microalgae triacylglycerol metabolism under heat stress

Autor: Jinshui Yang

Environ Res. 2022 May 15;208:112696. doi: 10.1016/j.envres.2022.112696. Epub 2022 Jan 10.

ABSTRACT

Microalgae are the largest CO2 fixer and O2 producer on the earth and occupy an increasingly important position in human life and production. Various environmental factors have a significant impact on the growth and metabolism of microalgae. As global warming intensifies, heat stress has become a crucial factor affecting the microalgae industry. However, till now, it has not been clear how microalgae sensed the temperature stress, transmitted stress signals and adjusted in intracellular metabolic pathways. In this study, the growth of microalgae Auxenochlorella protothecoides UTEX2341 was inhibited at 32 °C, but the single cell dry weight increased. The cell component analyses showed that both the carbohydrate and total protein content decreased significantly, while the lipid content increased by 158%. Meanwhile, the intracellular Ca2+ concentration increased continuously, with a maximum increase of 1.65 times. According to the transcriptome analyses, the up-regulation of Ca2+ influx channel protein mid1-complementing activity 1 (MCA1) gene and the down-regulation of efflux channel protein cation exchanger 1(CAX) and autoinhibited Ca2+-ATPase 1 (ACA1) genes in cytoplasmic membrane jointly facilitated the increase of Ca2+ in the cytoplasm. Coexpression network analysis indicated that the fluctuation of Ca2+ in the cytoplasm could activate the expression of transcription factors MYB3 and AP2-4 through calmodulin (CAM) and calcium-dependent protein kinase (CDPK), and then regulate glycerol-3-phosphate acyltransferases (GPAT) at the beginning of TAG synthesis and diacylglycerol acyltransferase (DGAT)/phospholipid: diacylglycerol acyltransferase (PDAT) in the last step of TAG synthesis. Furthermore, the addition of Ca2+ specific chelator BAPTA-AM inhibited the expression of GPAT, which was consistent with the decrease in microalgae lipid content. The results proved that Ca2+ participated in the regulation of microalgae TAG synthesis under heat stress, which provided a new view for the understanding of the microalgae lipid accumulation mechanism.

## Carbonate assisted lipid extraction and biodiesel production from wet microalgal biomass and recycling waste carbonate for CO(2) supply in microalgae cultivation

Autor: Ruolan Zhang

Sci Total Environ. 2021 Jul 20;779:146445. doi: 10.1016/j.scitotenv.2021.146445. Epub 2021 Mar 14.

ABSTRACT

High cost of microalgal biofuel is caused by all the steps in current technology, including cultivation, harvesting, lipid extraction, biofuel processing and wastewater and waste treatment. This study aims to systematically reduce these costs with one integrated process, in which carbonate is used for cell rupture, lipid extraction and biodiesel processing, and then recycled for CO2 absorption and carbon supply for a new round of algae cultivation. To reach this goal, carbonate-heating treatment with N, N' - dibutylurea which can enhance cell disruption were used for cell-wall breaking of wet Neochloris oleoabundans (UTEX 1185) biomass. Lipid extraction was fulfilled with carbonate/ethanol aqueous two phase extraction method and residual carbonate with wastewater from bottom phase was recycled to absorb CO2 to generate bicarbonate for algal cultivation with fresh medium. Taking into comprehensive consideration of cell disruption efficiency, partition coefficient, and lipid recovery, the condition of cell disruption and lipid extraction was set at 90 °C, 100 min reaction time, 1:7.5 DBU:H2O (w/w) ratio, 1:3 Na2CO3:H2O (w/w) ratio, and 9% (w/wT) ethanol concentration. The results showed that carbonate-heating treatment of wet N. oleoabundans biomass resulted in up to 90.7% cell disruption efficiency. The lipid recovery rate in carbonate/ethanol system was up to 97.9%, and the final biodiesel production was 1.30 times of that with Soxhlet method. Utilization of the waste broth after CO2 absorption with the content of 4% (v/vT) in the medium for new batch of algae cultivation resulted in biomass concentration of 1.68 g/L. The corresponding total fatty acids production was 0.35 g/L, which was 1.63 fold of that with fresh medium. This study firstly proved the feasibility of using carbonate for lipid extraction and biodiesel production and recycle waste carbonate for carbon re-supply during algae cultivation.

## Characterization of Novel Selected Microalgae for Antioxidant Activity and Polyphenols, Amino Acids, and Carbohydrates

Autor: Paula Santiago-Díaz

Mar Drugs. 2021 Dec 30;20(1):40. doi: 10.3390/md20010040.

ABSTRACT

The biochemical composition of three novel selected microalgae strains (Chlorophyta) was evaluated to confirm their potential possibilities as new sustainably produced biomass with nutritional, functional, and/or biomedical properties. Extracts from cultured Pseudopediastrum boryanum, Chloromonas cf. reticulata, and Chloroidium saccharophilum exhibited higher radical scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) when compared to butylated hydroxytoluene (BHT), but lower than butylated hydroxyanisole (BHA). Total phenolic compounds and amino acids were determined by newly developed RP-HPLC methods. Total phenolic contents, as µg g-1 of dry biomass, reached 27.1 for C. cf. reticulata, 26.4 for P. boryanum, and 55.8 for C. saccharophilum. Percentages of total analysed amino acids were 24.3, 32.1, and 18.5% of dry biomass, respectively, presenting high values for essential amino acids reaching 54.1, 72.6, and 61.2%, respectively. Glutamic acid was the most abundant free amino acid in all microalgae samples, followed by proline and lysine in C. saccharophilum and P. boryanum, and methionine and lysine in C. reticulata. Soluble carbohydrates in aqueous extracts ranged from 39.6 for C. saccharophilum to 49.3% for C. reticulata, increasing values to 45.1 for C. saccharophilum and 52.7% for P. boryanum in acid hydrolysates of dried biomass. Results confirmed the potential possibilities of these microalgae strains.

## Characterization of an aerated submerged hollow fiber ultrafiltration device for efficient microalgae harvesting

Autor: Franziska Ortiz Tena

Eng Life Sci. 2021 Sep 12;21(10):607-622. doi: 10.1002/elsc.202100052. eCollection 2021 Oct.

ABSTRACT

The present work characterizes a submerged aerated hollow fiber polyvinylidene fluorid (PVDF) membrane (0.03 µm) device (Harvester) designed for the ultrafiltration (UF) of microalgae suspensions. Commercial baker's yeast served as model suspension to investigate the influence of the aeration rate of the hollow fibers on the critical flux (CF, J c) for different cell concentrations. An optimal aeration rate of 1.25 vvm was determined. Moreover, the CF was evaluated using two different Chlorella cultures (axenic and non-axenic) of various biomass densities (0.8-17.5 g DW/L). Comparably high CFs of 15.57 and 10.08 L/m/2/h were measured for microalgae concentrations of 4.8 and 10.0 g DW/L, respectively, applying very strict CF criteria. Furthermore, the J c-values correlated (negative) linearly with the biomass concentration (0.8-10.0 g DW/L). Concentration factors between 2.8 and 12.4 and volumetric reduction factors varying from 3.5 to 11.5 could be achieved in short-term filtration, whereat a stable filtration handling biomass concentrations up to 40.0 g DW/L was feasible. Measures for fouling control (aeration of membrane fibers, periodic backflushing) have thus been proven to be successful. Estimations on energy consumption revealed very low energy demand of 17.97 kJ/m3 treated microalgae feed suspension (4.99 × 10-3 kWh/m3) and 37.83 kJ/kg treated biomass (1.05 × 10-2 kWh/kg), respectively, for an up-concentration from 2 to 40 g DW/L of a microalgae suspension.

## Characterization of isolated UV-C-irradiated mutants of microalga Chlorella vulgaris for future biofuel application

Autor: Jessa Dg Carino

Environ Dev Sustain. 2022 Jan 4:1-18. doi: 10.1007/s10668-021-02091-8. Online ahead of print.

ABSTRACT

Microalgae-based biofuel is considered as one of the most promising sources of alternative energy because it is sustainable and does not pose threats to the environment and food security. However, attempts in improving microalgal strains to attain the ideal characteristics for biofuel application are yet to unravel. In this study, random UV-C mutagenesis was employed to generate starch-deficient mutants of indigenous Chlorella vulgaris to enhance its productivity. Out of 872 colonies, two isolated mutants (cvm5 and cvm6) were isolated and showed significant increase in cell concentrations by > 1.47-fold and > 1.04-fold, respectively. However, mutant cells exhibited smaller in size which might contributed to the significant decrease in their biomass. Moreover, gathered data revealed that the total lipid content of cvm5 was enhanced significantly (75%, > 1.3-fold increase). Additionally, triacylglycerol (TAG) content of the said mutant constitutes 48% of the dry cell weight (DCW) while cvm6 consist of 41% of the DCW. These promising and novel findings suggest that the two generated and isolated mutants are good candidates for future commercial biofuel production, especially in the Philippines. In addition, these findings may contribute on the prior knowledge of the usage of UV-C for microalgal strain development.

## Chlorella vulgaris meets TiO2 NPs: Effective sorbent/photocatalytic hybrid materials for water treatment application

07.12.21 08:00

Autor: M Blosi

J Environ Manage. 2022 Feb 15;304:114187. doi: 10.1016/j.jenvman.2021.114187. Epub 2021 Dec 5.

ABSTRACT

A new class of bio-nano hybrid catalyst useable in downstream wastewater treatment was developed. We combined the sorption potentialities of Chlorella vulgaris microalgae with the photocatalytic properties of TiO2 NPs in order to investigate unexplored synergistic effects that could push the algal remediation technology toward a more promising cost-effective balance. We exploited non-living C. vulgaris, which keeps the biosorption properties of the living microalgae, but greatly enhancing the overall processability. C. vulgaris biomass was coupled with TiO2 NPs and the nanosols were then dried by means of a spray freeze drying (SFD) process able to produce highly reactive granules. A widespread physicochemical characterization supported the preparation and the performance evaluation, so highlighting the key-role of C. vulgaris/TiO2 interaction at the colloidal state. Heavy metal adsorption, tested for copper ions, and photocatalytic activity, assessed for Rhodamine B (RhB) photodegradation, were evaluated as key performances. The results pointed out a positive synergistic effect for hybrid samples consistent with the enhancement of metal biosorption which ranges from 103 mg g-1, for pristine C. vulgaris, to about 4000 mg g-1, when the biomass was coupled with the inorganic nanophase. The photocatalytic activity was well preserved with a complete RhB conversion after 1 h and even advanced in presence of SiO2NPs into the inorganic counterpart, so increasing the kinetic constant from 8.70 to 10.7 10-2 min-1. The results pave the way for the integration of these sorbent/photocatalytic hybrid materials into water remediation systems in an innovative sustainable design perspective.

## Combination of Synergic Enzymes and Ultrasounds as an Effective Pretreatment Process to Break Microalgal Cell Wall and Enhance Algal Oil Extraction

Autor: Cristina Blanco-Llamero

Foods. 2021 Aug 19;10(8):1928. doi: 10.3390/foods10081928.

ABSTRACT

Microalgal biomass is a sustainable source of bioactive lipids with omega-3 fatty acids. The efficient extraction of neutral and polar lipids from microalgae requires alternative extraction methods, frequently combined with biomass pretreatment. In this work, a combined ultrasound and enzymatic process using commercial enzymes Viscozyme, Celluclast, and Alcalase was optimized as a pretreatment method for Nannochloropsis gaditana, where the Folch method was used for lipid extraction. Significant differences were observed among the used enzymatic pretreatments, combined with ultrasound bath or probe-type sonication. To further optimize this method, ranges of temperatures (35, 45, and 55 °C) and pH (4, 5, and 8) were tested, and enzymes were combined at the best conditions. Subsequently, simultaneous use of three hydrolytic enzymes rendered oil yields of nearly 29%, showing a synergic effect. To compare enzymatic pretreatments, neutral and polar lipids distribution of Nannochloropsis was determined by HPLC-ELSD. The highest polar lipids content was achieved employing ultrasound-assisted enzymatic pretreatment (55 °C and 6 h), whereas the highest glycolipid (44.54%) and PE (2.91%) contents were achieved using Viscozyme versus other enzymes. The method was applied to other microalgae showing the potential of the optimized process as a practical alternative to produce valuable lipids for nutraceutical applications.

## Comparative Proteomics Reveals Evidence of Enhanced EPA Trafficking in a Mutant Strain of Nannochloropsis oculata

Autor: Wan Aizuddin Wan Razali

Front Bioeng Biotechnol. 2022 May 12;10:838445. doi: 10.3389/fbioe.2022.838445. eCollection 2022.

ABSTRACT

The marine microalga Nannochloropsis oculata is a bioproducer of eicosapentaenoic acid (EPA), a fatty acid. EPA is incorporated into monogalactosyldiacylglycerol within N. oculata thylakoid membranes, and there is a biotechnological need to remodel EPA synthesis to maximize production and simplify downstream processing. In this study, random mutagenesis and chemical inhibitor-based selection method were devised to increase EPA production and accessibility for improved extraction. Ethyl methanesulfonate was used as the mutagen with selective pressure achieved by using two enzyme inhibitors of lipid metabolism: cerulenin and galvestine-1. Fatty acid methyl ester analysis of a selected fast-growing mutant strain had a higher percentage of EPA (37.5% of total fatty acids) than the wild-type strain (22.2% total fatty acids), with the highest EPA quantity recorded at 68.5 mg/g dry cell weight, while wild-type cells had 48.6 mg/g dry cell weight. Label-free quantitative proteomics for differential protein expression analysis revealed that the wild-type and mutant strains might have alternative channeling pathways for EPA synthesis. The mutant strain showed potentially improved photosynthetic efficiency, thus synthesizing a higher quantity of membrane lipids and EPA. The EPA synthesis pathways could also have deviated in the mutant, where fatty acid desaturase type 2 (13.7-fold upregulated) and lipid droplet surface protein (LDSP) (34.8-fold upregulated) were expressed significantly higher than in the wild-type strain. This study increases the understanding of EPA trafficking in N. oculata, leading to further strategies that can be implemented to enhance EPA synthesis in marine microalgae.

## Continuous electrocoagulation of Chlorella vulgaris in a novel channel-flow reactor: A pilot-scale harvesting study

Autor: Simona Lucakova

Bioresour Technol. 2022 Mar 12;351:126996. doi: 10.1016/j.biortech.2022.126996. Online ahead of print.

ABSTRACT

The most frequently used method to harvest microalgae on an industrial scale is centrifugation, although this has very high energy costs. To reduce these costs, a continuous electrocoagulation process for harvesting Chlorella vulgaris was developed and tested using a pilot-scale 111 L working volume device consisting of an electrolyser with iron electrodes, aggregation channel and lamellar settler. The flow rate of the microalgal suspension through the device was 240 L/h. When using controlled cultivation and subsequent electrocoagulation, a high harvesting efficiency (above 85%), a low Fe contamination in the harvested biomass (<4 mg Fe/g dry biomass, a harvested biomass complied with legislative requirements for food) and significant energy savings were achieved. When comparing electrocoagulation and subsequent centrifugation with the use of centrifugation alone, energy savings were 80 % for a biomass harvesting concentration of 0.23 g/L. Electrocoagulation was thus proven to be a feasible pre-concentration method for harvesting microalgae.

## Cross-Linked Enzyme Aggregates and Their Application in Enzymatic Pretreatment of Microalgae: Comparison Between CLEAs and Combi-CLEAs

Autor: Cristina Blanco-Llamero

Front Bioeng Biotechnol. 2021 Dec 9;9:794672. doi: 10.3389/fbioe.2021.794672. eCollection 2021.

ABSTRACT

Carrier-free immobilization is a key process to develop efficient biocatalysts able to catalyze the cell wall degradation in microalgae where the traditional solid supports cannot penetrate. Thus, the insolubilization of commercial Celluclast®, Alcalase®, and Viscozyme® enzymes by carrier-free immobilization and their application in microalgae pretreatment was investigated. In this study, different precipitants at different ratios (ethanol, acetone, and polyethylene glycol 4000) were tested in the first part of the method, to establish the precipitation conditions. The screening of the best precipitant is needed as it depends on the nature of the enzyme. The best results were studied in terms of immobilization yield, thermal stability, and residual activity and were analyzed using scanning electron microscopy. Moreover, a novel strategy was intended including the three enzymes (combi-CLEAs) to catalyze the enzymatic degradation of Nannochloropsis gaditana microalgal cell wall in one pot. The carrier-free immobilized derivatives were 10 times more stable compared to soluble enzymes under the same. At the best conditions showed its usefulness in the pretreatment of microalgae combined with ultrasounds, facilitating the cell disruption and lipid recovery. The results obtained suggested the powerful application of these robust biocatalysts with great catalytic properties on novel and sustainable biomass such as microalgae to achieve cost-effective and green process to extract valuable bioactive compounds.

## Design and fabrication of box-type passive solar dryer (BTPSD) with thermal insulation material for valorizing biomass and neutral lipids of marine Chlorella vulgaris for biodiesel application

Autor: N Kalaiselvan

Sci Rep. 2022 Apr 11;12(1):6046. doi: 10.1038/s41598-022-09665-0.

ABSTRACT

The staggering rate of population growth has augmented the reliance on fossil fuel utilization, and it kindled the society to explore alternative and sustainable sources of energy. In this regard, biodiesel from microalgae came to the limelight; but crucial energy-consuming and expensive processes like cultivation, harvesting, and drying make the microalgal biodiesel unsustainable and economically unfeasible. To surpass these impediments, in this research work, a low-cost box-type passive solar dryer (BTPSD) is designed and fabricated with zero energy consumption mode and compared with conventional hot air oven for drying the biomass, neutral lipids of the marine microalga Chlorella vulgaris for biodiesel application. The onset of the work, BTPSD with 2 cm thickness of glass wool as TIM (thermal insulation material), 4 cm TIM thickness and no TIM was simulated for thermal storage behaviour using ANSYS FLUENT 19.2 Computational Fluid Dynamics tool and based on the results, 4 cm TIM thickness was chosen for experimentation. The time taken by BTPSD and hot air oven to remove the moisture from algal biomass is 3 and 2 h, respectively, whereas for neutral lipids drying, it was 4 and 3.5 h, respectively. Though there is a little difference in drying time, neutral lipid and FAME content from both drying systems are tantamount, i.e., ~ 12% neutral lipid and 95% FAME. Further, the percentage of vital fatty acids identified from BTPSD and hot air oven methods are almost similar, i.e., C16:0 (23.4%), C18:1 (14.3%), C18:3 (11.42%), C18:1 (9.22%). Though the time taken for valorizing biomass and neutral lipids of C. vulgaris by BTPSD is slightly longer than hot air oven, low energy consumption and cost-effectiveness make the BTPSD a promising system to scale down the microalgal biodiesel production cost significantly.

## Determination of veterinary drugs in microalgae biomass from photobioreactors fed with piggery wastewater

Autor: Rebeca López-Serna

Chemosphere. 2022 Jan;287(Pt 1):132076. doi: 10.1016/j.chemosphere.2021.132076. Epub 2021 Aug 30.

ABSTRACT

Concentration data of veterinary drugs in microalgae biomass collected from photobioreactors fed with piggery wastewaters are presented for the first time in this work. To this aim, a QuEChERS methodology and an ultrasound-assisted solid-liquid extraction have been assessed as sample preparation procedures with the purpose of determining 20 veterinary drugs, mainly antibiotics of different physico-chemical properties in addition to dexamethasone, fenbendazole and progesterone. Some critical operation parameters of the QuEChERS procedure were optimized by an experimental design but tetracycline, oxytetracycline, doxycycline, marbofloxacin and ciprofloxacin were not detected by the QuEChERS sample preparation. The use of a longer and thorough approach, a solid-liquid extraction with water/methanol in presence of primary secondary amine as a clean-up agent followed by solid-phase extraction on Oasis HLB cartridges, is recommended to monitor all intended analytes. The determination in extracts is carried out by ultra-high performance liquid chromatography-tandem mass spectrometry in selected reaction monitoring mode. Limits of detection about 0.2-42 ng per g of lyophilized microalgae sample, and repeatabilities about 6-46% (n = 5, RSDs) are reached. The solid-liquid extraction method was applied to microalgae biomass samples collected from a photobioreactor. Nine drugs were detected in the samples at relatively low concentration and a proportional relationship between the found concentrations and the octanol/water partition coefficients of the drugs has been outlined. Moreover, a linear ratio between the concentrations measured in biomass and effluent has been observed for most of the drugs.

## Developing a Chromochloris zofingiensis Mutant for Enhanced Production of Lutein under CO2 Aeration

Autor: Yuanyuan Ren

Mar Drugs. 2022 Mar 7;20(3):194. doi: 10.3390/md20030194.

ABSTRACT

Microalgae are competitive and commercial sources for health-benefit carotenoids. In this study, a Chromochloris zofingiensis mutant (Cz-pkg), which does not shut off its photosystem and stays green upon glucose treatment, was generated and characterized. Cz-pkg was developed by treating the algal cells with a chemical mutagen as N-methyl-N'-nitro-N-nitrosoguanidine and followed by a color-based colony screening approach. Cz-pkg was found to contain a dysfunctional cGMP-dependent protein kinase (PKG). By cultivated with CO2 aeration under mixotrophy, the mutant accumulated lutein up to 31.93 ± 1.91 mg L-1 with a productivity of 10.57 ± 0.73 mg L-1 day-1, which were about 2.5- and 8.5-fold of its mother strain. Besides, the lutein content of Cz-pkg could reach 7.73 ± 0.52 mg g-1 of dry weight, which is much higher than that of marigold flower, the most common commercial source of lutein. Transcriptomic analysis revealed that in the mutant Cz-pkg, most of the genes involved in the biosynthesis of lutein and chlorophylls were not down-regulated upon glucose addition, suggesting that PKG may regulate the metabolisms of photosynthetic pigments. This study demonstrated that Cz-pkg could serve as a promising strain for both lutein production and glucose sensing study.

## Development of an Efficient Extraction Method for Harvesting Gymnodimine-A from Large-Scale Cultures of Karenia selliformis

Autor: Zhixuan Tang

Toxins (Basel). 2021 Nov 10;13(11):793. doi: 10.3390/toxins13110793.

ABSTRACT

Gymnodimine-A (GYM-A) is a fast-acting microalgal toxin and its production of certified materials requires an efficient harvesting technology from the large-scale cultures of toxigenic microalgae. In this study the recoveries of GYM-A were compared between several liquid-liquid extraction (LLE) treatments including solvents, ratios and stirring times to optimize the LLE technique for harvesting GYM-A from Karenia selliformis cultures, of which the dichloromethane was selected as the extractant and added to microalgal cultures at the ratio 55 mL L-1 (5.5%, v/v). The recovery of GYM-A obtained by the LLE technique was also compared with filtration and centrifugation methods. The stability of GYM-A in culture media were also tested under different pH conditions. Results showed that both the conventional filter filtration and centrifugation methods led to fragmentation of microalgal cells and loss of GYM-A in the harvesting processes. A total of 5.1 μg of GYM-A were obtained from 2 L of K. selliformis cultures with a satisfactory recovery of 88%. Interestingly, GYM-A obviously degraded in the culture media with the initial pH 8.2 and the adjusted pH of 7.0 after 7 days, but there was no obvious degradation in the acidic medium at pH 5.0. Therefore, the LLE method developed here permits the collection of large-volume cultures of K. selliformis and the high-efficiency extraction of GYM-A. This work provides a simple and valuable technique for harvesting toxins from large-scale cultures of GYM-producing microalgae.

## Distinctive correlations between cell concentration and cell size to microalgae biomass under increasing carbon dioxide

Autor: Yi An Lim

Bioresour Technol. 2022 Mar;347:126733. doi: 10.1016/j.biortech.2022.126733. Epub 2022 Jan 21.

ABSTRACT

Carbon capture and storage (CCS) via microalgae cultivations is getting renewed interest as climate change mitigation effort, owing to its excellent photosynthetic and CO2 fixation capability. Microalgae growth is monitored based on their biomass, cell concentrations and cell sizes. The key parametric relationships on microalgae growth under CO2 are absent in previous studies and this inadequacy hampers the design and scale-up of microalgae-based CCS. In this study, three representative microalgae species, Chlorella, Nostoc and Chlamydomonas, were investigated for establishing key correlations of cell concentrations and sizes towards their biomass fluctuations under CO2 influences of 0% to 20% volume ratios (v/v). This revealed that Chlorella and Chlamydomonas cell concentrations significantly contributed towards increasing biomass concentration under CO2 elevations. Chlorella and Nostoc cell sizes were enhanced at 20% (v/v). These findings provided new perspectives on growth responses under increasing CO2 treatment, opening new avenues on CCS schemes engineering designs and biochemical production.

## Effect of different wavelengths of LED light on the growth, chlorophyll, β-carotene content and proximate composition of Chlorella ellipsoidea

Autor: Arpan Baidya

Heliyon. 2021 Dec 3;7(12):e08525. doi: 10.1016/j.heliyon.2021.e08525. eCollection 2021 Dec.

ABSTRACT

Chlorella ellipsoidea is a freshwater green microalga that has great prospect for the sustainable development of aquaculture industry. Microalgae require optimal lighting conditions for efficient photosynthesis. The key to cost-effective algal biomass production is to optimize algae growth conditions. This study aimed to investigate the effects of various wavelengths viz. white (380-750 nm), green (510 nm), blue (475 nm), and red (650 nm) light-emitting diodes (LEDs) on the growth, pigment content (chlorophyll-a, chlorophyll-b, and β-carotene), and proximate composition of C. ellipsoidea with a photoperiod of 12 h:12 h light: dark cycle under indoor environmental conditions. C. ellipsoidea was cultured in Bold's Basal Medium for 18 days. The cell density (125.36×105 cells ml-1), cell dry weight (58.9 ± 4.57 mg L-1), optical density (1.66 ± 0.08 g L-1), chlorophyll-a (7.31 ± 0.04 μg ml-1), chlorophyll-b (2.73 ± 0.13 μg ml-1), and β-carotene (0.39 ± 0.04 μg ml-1) content of C. ellipsoidea were significantly ($P < 0.05$) higher at 15th-day when cultured under blue LED light exposure. Significantly lower growth and nutritional values were obtained under red LED light exposure compared to the control and other LEDs spectra. In Pearson correlation analysis, the cell density and cell dry weight values showed a strong positive correlation with the values of pigment contents of C. ellipsoidea in all the treatments. The LEDs light spectra showed significant effects on proximate composition of C. ellipsoidea. Protein and lipid contents of C. ellipsoidea were significantly higher in blue LED growth conditions compared to white, green, and red LEDs. C. ellipsoidea cells were 3-7.04 μm in size and the maximum area of the cell was 38.94 μm2 in blue LED treatment. Results of this study demonstrated that blue LED light spectra was the most suitable condition to induce nutritionally rich biomass production of C. ellipsoidea, which can be used as a potential source of fish feed towards sustainable aquaculture.

## Effective Two-Stage Heterotrophic Cultivation of the Unicellular Green Microalga Chromochloris zofingiensis Enabled Ultrahigh Biomass and Astaxanthin Production

Autor: Qiaohong Chen

Front Bioeng Biotechnol. 2022 Feb 24;10:834230. doi: 10.3389/fbioe.2022.834230. eCollection 2022.

ABSTRACT

Chromochloris zofingiensis has obtained particular interest as a promising candidate for natural astaxanthin production. In this study, we established a two-stage heterotrophic cultivation process, by using which both the growth of C. zofingiensis and astaxanthin accumulation are substantially enhanced. Specifically, the ultrahigh biomass concentration of 221.3 g L-1 was achieved under the optimum culture conditions in 7.5 L fermenter during 12 days. When scaled-up in the 500 L fermentor, the biomass yield reached 182.3 g L-1 in 9 days, while the astaxanthin content was 0.068% of DW. To further promote astaxanthin accumulation, gibberellic Acid-3 (GA3) was screened from a variety of phytohormones and was combined with increased C/N ratio and NaCl concentration for induction. When C. zofingiensis was grown with the two-stage cultivation strategy, the astaxanthin yield reached 0.318 g L-1, of which the biomass yield was 235.4 g L-1 and astaxanthin content was 0.144% of DW. The content of the total fatty acids increased from 23 to 42% of DW simultaneously. Such an astaxanthin yield was 5.4-fold higher than the reported highest record and surpassed the level of Haematococcus pluvialis. This study demonstrated that heterotrophic cultivation of C. zofingiensis is competitive for industrial astaxanthin production.

## Enhanced β-carotene and Biomass Production by Induced Mixotrophy in Dunaliella salina across a Combined Strategy of Glycerol, Salinity, and Light

Autor: Willian Capa-Robles

Metabolites. 2021 Dec 13;11(12):866. doi: 10.3390/metabo11120866.

ABSTRACT

Current mixotrophic culture systems for Dunaliella salina have technical limitations to achieve high growth and productivity. The purpose of this study was to optimize the mixotrophic conditions imposed by glycerol, light, and salinity that lead to the highest biomass and β-carotene yields in D. salina. The combination of 12.5 mM glycerol, 3.0 M salinity, and 50 µmol photons m-2 s-1 light intensity enabled significant assimilation of glycerol by D. salina and consequently enhanced growth (2.1 × 106 cell mL-1) and β-carotene accumulation (4.43 pg cell-1). The saline and light shock induced the assimilation of glycerol by this microalga. At last stage of growth, the increase in light intensity (300 µmol photons m-2 s-1) caused the β-carotene to reach values higher than 30 pg cell-1 and tripled the β-carotene values obtained from photoautotrophic cultures using the same light intensity. Increasing the salt concentration from 1.5 to 3.0 M NaCl (non-isosmotic salinity) produced higher growth and microalgal β-carotene than the isosmotic salinity 3.0 M NaCl. The mixotrophic strategy developed in this work is evidenced in the metabolic capability of D. salina to use both photosynthesis and organic carbon, viz., glycerol that leads to higher biomass and β-carotene productivity than that of an either phototrophic or heterotrophic process alone. The findings provide insights into the key role of exogenous glycerol with a strategic combination of salinity and light, which evidenced unknown roles of this polyol other than that in osmoregulation, mainly on the growth, pigment accumulation, and carotenogenesis of D. salina.

## Enhancement of protein production using synthetic brewery wastewater by Haematococcus pluvialis

Autor: Siao Ming Yap

J Biotechnol. 2022 Mar 21:S0168-1656(22)00056-6. doi: 10.1016/j.jbiotec.2022.03.008. Online ahead of print.

ABSTRACT

Microalgae is a sustainable protein source that has been widely applied in animal feeds, functional foods, pharmaceutical, and cosmeceutical industries. Waste products could be a potential cost-saving and nutrient-rich substrate in the cultivation of microalgae for protein production. This study aims to investigate the cultivation condition of Haematococcus pluvialis for protein synthesis using synthetic brewery wastewater (BW). H. pluvialis was cultivated in the Bold's Basal Medium (BBM) mixed with synthetic BW at different concentrations. Various cultivation conditions including brewer's spent grain hydrolysate (BSGH) concentrations, pH, and light sources were studied. The molecular weight, amino acids profile and antioxidant activity of synthesized protein were determined. Fed-batch cultivation using different percentages of fresh medium replacement for enhancing protein production was investigated. The 20% fed-batch cultivation reached 27 ×105 ± 0.42 cells/mL, and 4-fold of the protein content of 64.93 ± 5.30% of dry weight was recorded on day-13. Seven essential amino acids (lysine, threonine, histidine, phenylalanine, isoleucine, leucine, methionine) were produced in fed-batch cultivation. Red LED obtained the highest DPPH radical scavenging activity of 27.47 ± 0.98%. The findings suggested that BW is a promising substrate in the cultivation of H. pluvialis to commercially produce protein for numerous industrial applications.

## Enhancing nitrogen removal in mature landfill leachate by mixed microalgae through elimination of inhibiting factors

Autor: Zhangbao Wang

Sci Total Environ. 2022 Mar 13;828:154530. doi: 10.1016/j.scitotenv.2022.154530. Online ahead of print.

ABSTRACT

Nitrogen removal from landfill leachate (LL) using microalgae is a promising method and can realize CO2 mitigation. But the performances are usually inhibited by high chromaticity, high free ammonia (FAN) and some complex macro molecular organic matter (MOM) in the LL. To achieve efficient nitrogen removal from LL, this study firstly pretreated the mature LL with ozone, decolorizer and activated sludge (AS) respectively, and then inoculated with mixed microalgae. The results showed that the synergistic effect of ozonation and microalgae was the best among the three, with 99.7% ammonia removal, 0.77 g/L (dry weight) microalgae biomass, and a maximum growth rate of 160 mg/L/d. Ozonation pretreatment significantly reduced the chromaticity and macromolecular organic matter of LL, with the chromaticity reduced from 2225 to 225 times and the 3D fluorescence intensity representing MOM reduced from 4089 a.u. to 986.1 a.u.. And it was found that the mixed microalgae grown after pretreatment by three different methods all were mostly Chlorella and very few Microcystis, and the density of microalgal populations (number of cells per unit volume) after ozonation was up to 10,650 cells/μL. This work provides a feasible and an economical way to remove ammonia nitrogen (NH+ 4-N) from landfill leachate.

## Enriching table eggs with n-3 polyunsaturated fatty acids through dietary supplementation with the phototrophically grown green algae Nannochloropsis limnetica: effects of microalgae on nutrient retention, performance, egg characteristics and health parameters

Autor: A J W Mens

Poult Sci. 2022 Mar 24;101(6):101869. doi: 10.1016/j.psj.2022.101869. Online ahead of print.

ABSTRACT

The fatty acid content of microalgae, especially the high content of omega-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), could enrich eggs when fed to laying hens. Moreover, the properties and bioactive components of omega-3 fatty acids could positively influence the health and production performance of laying hens. In this study, the effects of dried Nannochloropsis limnetica inclusions in diets on yolk omega-3 fatty acid content, laying hen performance, nutrient retention, intestinal morphometry and systemic inflammatory markers were measured. A total of 240 twenty-five-wk-old laying hens were randomly assigned to 5 treatments distributed among 30 pens. Treatment A received the reference diet, while diets in treatments B, C, and D contained the control diet with 1, 2, and 3% microalgae added, respectively. In treatment E, a portion of ingredients of the control diet was replaced with rapeseed meal to induce a mild nutritional challenge, along with an inclusion of 3% microalgae. Compared to the control group the rate of lay increased by approximately 5% ($P = 0.039$) when birds were fed 2 or 3% microalgae. Furthermore, inclusion of 2 and 3% microalgae resulted in higher feed intake compared to the control group (126, 125, and 119 g/hen/d respectively; $P = 0.001$). Other performance parameters such as nutrient retention and egg characteristics were not affected by the dietary treatments. The EPA and DHA content of the yolk increased with increasing microalgae inclusion level ($P < 0.001$). A 2% algal inclusion resulted in 58.3 (EPA) and 603 (DHA) mg per 100 g dry yolk, respectively. Plasma haptoglobin levels of laying hens in both treatments receiving 3% microalgae were almost 3 times lower compared to the control group (1.25 and 1.62 vs. 5.60; $P < 0.001$), regardless of the inclusion of rapeseed in the diet. Based on these results, it can be

concluded that the inclusion of N. limnetica enriches the egg yolk without negatively affecting the performance of laying hens and egg characteristics. Due to the positive effect on feed intake, microalgae in the diet provide nutritional benefits for laying hens. However, the positive effects of microalgae, especially on the health of laying hens, warrants further research.

## Exploring kinetics, removal mechanism and possible transformation products of tigecycline by Chlorella pyrenoidosa

Autor: Xueqing Zhong

Sci Total Environ. 2022 Apr 15;817:152988. doi: 10.1016/j.scitotenv.2022.152988. Epub 2022 Jan 11.

ABSTRACT

The accumulation of antibiotics in wastewater leads to broad antibiotic resistance, threating human health. Microalgae have been receiving attention due to their ability to remove antibiotics from wastewater. Tigecycline (TGC) is a broad-spectrum glycylcycline antibiotic. It has not been investigated for removal by microalgae. The removal kinetics of TGC by Chlorella pyrenoidosa were evaluated under different initial dry cell densities, TGC concentrations, temperatures and light intensity conditions. Approximately 90% of TGC could be removed when the TGC concentration was 10 mg·L-1 and the initial dry cell density was more than 0.2 g·L-1. A low value of TGC per g dry cell weight ratio led to a high removal efficiency of TGC. The initial dry cell density of microalgae was also critical for the removal of TGC. A high initial dry cell density is better than a low initial dry cell density to remove TGC when the ratio of the TGC concentration to dry cell weight are the same at the beginning of the cultivation. The removal mechanisms were investigated. Photolysis was a slow process that did not lead to removal at the beginning. Adsorption, hydrolysis, photolysis and biodegradation by microalgae were the main contributors to the removal of TGC. TGC was easily hydrolyzed under high -temperature conditions. Three transformation products of TGC by microalgae were identified. The stability of TGC was evaluated in water and salt solutions of citric acid, K2HPO4·3H2O and ferric ammonium citrate. TGC was stable in ultrapure water and citric acid solution. TGC was hydrolyzed in K2HPO4·3H2O and ferric ammonium citrate solutions.

## Extraction of astaxanthin from Haematococcus pluvialis with hydrophobic deep eutectic solvents based on oleic acid

Autor: Walter Pitacco

Food Chem. 2022 Jun 15;379:132156. doi: 10.1016/j.foodchem.2022.132156. Epub 2022 Jan 15.

ABSTRACT

Three novel hydrophobic deep eutectic solvents (DESs) based on oleic acid and terpenes (thymol, dl-menthol, and geraniol) were prepared, characterized, and used to extract astaxanthin from the microalga Haematococcus pluvialis without any pre-treatment of the cells. The three DES were composed of Generally Recognized As Safe (GRAS) and edible ingredients. All the tested DESs gave astaxanthin recovery values of about 60 and 30% in 6 h if applied on freeze-dried biomass or directly on algae culture, respectively. The carotenoid profile was qualitatively identical to what was obtained by using traditional organic solvents, regardless of the DES used; the monoesters of astaxanthin with C18-fatty acids were the main compounds found in all the carotenoid extracts. The thymol:oleic acid DES (TAO) could preserve astaxanthin content after prolonged oxidative stress (40% of the astaxanthin initially extracted was still present after 13.5 h of light exposure), thanks to the superior antioxidant properties of thymol. The capacity of improving astaxanthin stability combined with the intrinsic safety and edibility of the DES

components makes the formulation astaxanthin-TAO appealing for the food ingredients/additives industry.

## Feasibility of Utilizing Wastewaters for Large-Scale Microalgal Cultivation and Biofuel Productions Using Hydrothermal Liquefaction Technique: A Comprehensive Review

Autor: Sourav Kumar Bagchi

Front Bioeng Biotechnol. 2021 Nov 19;9:651138. doi: 10.3389/fbioe.2021.651138. eCollection 2021.

ABSTRACT

The two major bottlenecks faced during microalgal biofuel production are, (a) higher medium cost for algal cultivation, and (b) cost-intensive and time consuming oil extraction techniques. In an effort to address these issues in the large scale set-ups, this comprehensive review article has been systematically designed and drafted to critically analyze the recent scientific reports that demonstrate the feasibility of microalgae cultivation using wastewaters in outdoor raceway ponds in the first part of the manuscript. The second part describes the possibility of bio-crude oil production directly from wet algal biomass, bypassing the energy intensive and time consuming processes like dewatering, drying and solvents utilization for biodiesel production. It is already known that microalgal drying can alone account for ~30% of the total production costs of algal biomass to biodiesel. Therefore, this article focuses on bio-crude oil production using the hydrothermal liquefaction (HTL) process that converts the wet microalgal biomass directly to bio-crude in a rapid time period. The main product of the process, i.e., bio-crude oil comprises of C16-C20 hydrocarbons with a reported yield of 50-65 (wt%). Besides elucidating the unique advantages of the HTL technique for the large scale biomass processing, this review article also highlights the major challenges of HTL process such as update, and purification of HTL derived bio-crude oil with special emphasis on deoxygenation, and denitrogenation problems. This state of art review article is a pragmatic analysis of several published reports related to algal crude-oil production using HTL technique and a guide towards a new approach through collaboration of industrial wastewater bioremediation with rapid one-step bio-crude oil production from chlorophycean microalgae.

## Freeze-dried Nannochloropsis oceanica biomass protects eicosapentaenoic acid (EPA) from metabolization in the rumen of lambs

Autor: Ana C M Vítor

Sci Rep. 2021 Nov 8;11(1):21878. doi: 10.1038/s41598-021-01255-w.

ABSTRACT

Eicosapentaenoic acid (EPA) from freeze-dried biomass of Nannochloropsis oceanica microalgae resists ruminal biohydrogenation in vitro, but in vivo demonstration is needed. Therefore, the present study was designed to test the rumen protective effects of N. oceanica in lambs. Twenty-eight lambs were assigned to one of four diets: Control (C); and C diets supplemented with: 1.2% Nannochloropsis sp. oil (O); 12.3% spray-dried N. oceanica (SD); or 9.2% N. oceanica (FD), to achieve 3 g EPA /kg dry matter. Lambs were slaughtered after 3 weeks and digestive contents and ruminal wall samples were collected. EPA concentration in the rumen of lambs fed FD was about 50% higher than lambs fed SD or O diets. Nevertheless, the high levels of EPA in cecum and faeces of animals fed N. oceanica biomass, independently of the drying method, suggests that EPA was not completely released and absorbed in the small intestine. Furthermore, supplementation with EPA sources also affected the ruminal biohydrogenation of C18 fatty acids, mitigating the shift from the t10 biohydrogenation pathways to the t11 pathways compared to the

Control diet. Overall, our results demonstrate that FD N. oceanica biomass is a natural rumen-protected source of EPA to ruminants.

## Growth under Different Trophic Regimes and Synchronization of the Red Microalga Galdieria sulphuraria

Autor: Vít Náhlík

Biomolecules. 2021 Jun 24;11(7):939. doi: 10.3390/biom11070939.

ABSTRACT

The extremophilic unicellular red microalga Galdieria sulphuraria (Cyanidiophyceae) is able to grow autotrophically, or mixo- and heterotrophically with 1% glycerol as a carbon source. The alga divides by multiple fission into more than two cells within one cell cycle. The optimal conditions of light, temperature and pH (500 µmol photons m-2 s-1, 40 °C, and pH 3; respectively) for the strain Galdieria sulphuraria (Galdieri) Merola 002 were determined as a basis for synchronization experiments. For synchronization, the specific light/dark cycle, 16/8 h was identified as the precondition for investigating the cell cycle. The alga was successfully synchronized and the cell cycle was evaluated. G. sulphuraria attained two commitment points with midpoints at 10 and 13 h of the cell cycle, leading to two nuclear divisions, followed subsequently by division into four daughter cells. The daughter cells stayed in the mother cell wall until the beginning of the next light phase, when they were released. Accumulation of glycogen throughout the cell cycle was also described. The findings presented here bring a new contribution to our general understanding of the cell cycle in cyanidialean red algae, and specifically of the biotechnologically important species G. sulphuraria.

## Guidance for formulating ingredients/products from Chlorella vulgaris and Arthrospira platensis considering carotenoid and chlorophyll bioaccessibility and cellular uptake

Autor: Pricila P Nass

Food Res Int. 2022 Jul;157:111469. doi: 10.1016/j.foodres.2022.111469. Epub 2022 Jun 6.

ABSTRACT

This study aimed to investigate the impact of different microalgal matrices on the bioaccessibility and uptake by Caco-2 cells of carotenoids and chlorophylls. In this way, the microalgal ingredients/products (whole dry biomass [WDB], whole ultrasonicated paste [WUP], and liposoluble pigment emulsion [LPE]) obtained from Chlorella vulgaris and Arthrospira platensis were submitted to in vitro simulated digestion. Apical uptake of pigments in micelles generated during the simulated digestion by Caco-2 human intestinal cells was determined. The influence of simulated digestion on carotenoid and chlorophyll stability and bioaccessibility was assessed by HPLC-PDA-MS/MS and the carotenoids and chlorophylls' bioaccessibility and cellular uptake were shown to be boosted according to the matrix (LPE > WUP > WDB). Our findings showed that Chlorella vulgaris and Arthrospira platensis could be considered in formulations when carotenoids and chlorophylls are the target molecules in the ingredients/products.

## Immune Status and Hepatic Antioxidant Capacity of Gilthead Seabream Sparus aurata Juveniles Fed Yeast and Microalga Derived beta-glucans

Autor: Bruno Reis

Mar Drugs. 2021 Nov 23;19(12):653. doi: 10.3390/md19120653.

ABSTRACT

This work aimed to evaluate the effects of dietary supplementation with β-glucans extracted from yeast (Saccharomyces cerevisiae) and microalga (Phaeodactylum tricornutum) on gene expression, oxidative stress biomarkers and plasma immune parameters in gilthead seabream (Sparus aurata) juveniles. A practical commercial diet was used as the control (CTRL), and three others based on CTRL were further supplemented with different β-glucan extracts. One was derived from S. cerevisiae (diet MG) and two different extracts of 21% and 37% P. tricornutum-derived β-glucans (defined as Phaeo21 and Phaeo37), to give a final 0.06% β-glucan dietary concentration. Quadruplicate groups of 95 gilthead seabream (initial body weight: 4.1 ± 0.1 g) were fed to satiation three times a day for 8 weeks in a pulse-feeding regimen, with experimental diets intercalated with the CTRL dietary treatment every 2 weeks. After 8 weeks of feeding, all groups showed equal growth performance and no changes were found in plasma innate immune status. Nonetheless, fish groups fed β-glucans supplemented diets showed an improved anti-oxidant status compared to those fed CTRL at both sampling points (i.e., 2 and 8 weeks). The intestinal gene expression analysis highlighted the immunomodulatory role of Phaeo37 diet after 8 weeks, inducing an immune tolerance effect in gilthead seabream intestine, and a general down-regulation of immune-related gene expression. In conclusion, the results suggest that the dietary pulse administration of a P. tricornutum 37% enriched-β-glucans extract might be used as a counter-measure in a context of gut inflammation, due to its immune-tolerant and anti-oxidative effects.

## Improving carbohydrate accumulation in Chlamydomonas debaryana induced by sulfur starvation using response surface methodology

Autor: Karima Tazi

Environ Sci Pollut Res Int. 2021 Nov 24. doi: 10.1007/s11356-021-17583-3. Online ahead of print.

ABSTRACT

Most methods that promote carbohydrate production negatively affect cell growth and microalgal biomass production. This study explores, in a two-stage cultivation strategy, in Chlamydomonas debaryana the optimization of certain culture conditions for high carbohydrate production without loss of biomass. In the first stage, the interaction between sodium bicarbonate supplementation, aeration, and different growth periods was optimized using the response surface methodology (RMS). The 3-factor Box-Behnken design (BBD) was applied, and a second-order polynomial regression analysis was used to analyze the experimental data. The results showed that 0.45 g L-1 of sodium bicarbonate combined with a good aerated agitation (0.6 L min-1) and a cultivation period of 18 days are optimal to produce 5.02 g L-1 of biomass containing 43% of carbohydrates.Under these optimized growth conditions, accumulation of carbohydrates was studied using different modes of nutritional stress. The results indicated that carbohydrate content was improved and the maximum accumulation (about 60% of the dry weight) was recorded under sulfur starvation with only a 14% reduction in biomass as compared to control. This study showed promising results as to biomass production and carbohydrate yield by the microalgae C. debaryana in view of production of third-generation biofuels.

## Improving the content of high value compounds in Nordic Desmodesmus microalgal strains

Autor: Sanjeet Mehariya

Bioresour Technol. 2022 Sep;359:127445. doi: 10.1016/j.biortech.2022.127445. Epub 2022 Jun 16.

ABSTRACT

Nordic Desmodesmus microalgal strains (2-6) and (RUC-2) were exposed to abiotic stress (light and salt) to enhance lipids and carotenoids. The biomass output of both strains increased by more than 50% during light stress of 800 µmol m-2 s-1 compared to control light. The biomass of Desmodesmus sp. (2-6) contained most lipids (15% of dry weight) and total carotenoids (16.6 mg g-1) when grown at moderate light stress (400 µmol m-2 s-1), which further could be enhanced up to 2.5-fold by salinity stress. Desmodesmus sp. (RUC-2) exhibited maximal lipid (26.5%) and carotenoid (43.8 mg L-1) content at light intensities of 400 and 100 µmol m-2 s-1, respectively. Salinity stress stimulated lipid accumulation by 39%. Nordic Desmodesmus strains therefore are not only able to tolerate stress conditions, but their biomass considerably improves under stress. These strains have high potential to be used in algal bio-factories on low-cost medium like Baltic seawater.

## Influence of Carbon Sources on Biomass and Biomolecule Accumulation in Picochlorum sp. Cultured under the Mixotrophic Condition

Autor: Rahul Kumar Goswami

Int J Environ Res Public Health. 2022 Mar 19;19(6):3674. doi: 10.3390/ijerph19063674.

ABSTRACT

The major downfalls of the microalgal biorefinery are low volume of high value product accumulation, low biomass productivity and high cultivation costs. Here, we aimed to improve the biomass productivity of the industrially relevant Picochlorum sp. BDUG 100241 strain. The growth of Picochlorum sp. BDUG 100241 was investigated under different cultivations conditions, including photoautotrophic (with light), mixotrophic (1% glucose, with light) and heterotrophic (1% glucose, without light). Among them, Picochlorum sp. BDUG100241 showed the highest growth in the mixotrophic condition. Under different (1%) carbon sources' supplementation, including glucose, sodium acetate, glycerol, citric acid and methanol, Picochlorum sp. BDUG100241 growth was tested. Among them, sodium acetate was found to be most suitable carbon source for Picochlorum sp. BDUG 100241 growth, biomass (1.67 ± 0.18 g/L) and biomolecule productivity. From the different concentrations of sodium acetate (0, 2.5, 5.0, 7.5 and 10 g/L) tested, the maximum biomass production of 2.40 ± 0.20 g/L with the biomass productivity of 95 ± 5.00 mg/L/d was measured from 7.5 g/L in sodium acetate. The highest total lipid (53.50 ± 1.70%) and total carotenoids (0.75 ± 0.01 µg/mL) contents were observed at the concentration of 7.5 g/L and 5.0 g/L of sodium acetate as a carbon source, respectively. In conclusion, the mixotrophic growth condition containing 7.5 g/L of sodium acetate showed the maximum biomass yield and biomolecule accumulation compared to other organic carbon sources.

## Influence of Different Light Sources on the Biochemical Composition of Arthrospira spp. Grown in Model Systems

Autor: Massimo Milia

Foods. 2022 Jan 29;11(3):399. doi: 10.3390/foods11030399.

ABSTRACT

Arthrospira platensis and Arthrospira maxima are prokaryotic microalgae commercially marketed as spirulina. The pigments extracted from these algae are widely used for cosmetic and nutraceutical applications. This work aimed to evaluate the influence of three light-emitting lamps (white, orange and blue) on the growth and biomass composition of two strains of A. platensis (M2 and M2M) and one of A. maxima. The obtained data show strain- and light-dependent responses of the microalgae. In addition, white and orange lights led to a similar overall effect by increasing the levels of chlorophyll a and carotenoids. However, exposure to orange light resulted in the highest dry weight (5973.3 mg L-1 in M2M), whereas white light stimulated an increase in the carbohydrate fraction (up to 42.36 g 100 g-1 in A. maxima). Conversely, blue light led to a constant increase in the concentration of phycocyanin (14 g 100 g-1 in A. maxima) and a higher content of proteins in all strains. These results provide important environmental information for modulating the growth of different spirulina strains, which can be used to address the synthesis of biochemical compounds of strategic importance for the development of new nutraceutical foods.

## Influence of Irradiance and Wavelength on the Antioxidant Activity and Carotenoids Accumulation in Muriellopsis sp. Isolated from the Antofagasta Coastal Desert

Autor: Daniela Diaz-MacAdoo

Molecules. 2022 Apr 8;27(8):2412. doi: 10.3390/molecules27082412.

ABSTRACT

Microalgae are a valuable natural resource for a variety of biocompounds such as carotenoids. The use of different light spectra and irradiance has been considered as a promising option to improve the production of these compounds. The objective of this study was to evaluate the influence of different wavelengths (white, red, and blue) and irradiances (80 and 350 µmol photons/m2/s) on the photosynthetic state, total carotenoids and lutein productivity (HPLC), lipids (Nile red method) and antioxidant activity (DPPH) of the microalgae Muriellopsis sp. (MCH-35). This microalga, which is a potential source of lutein, was isolated from the coastal desert of Antofagasta, Chile, and adapted to grow in seawater. The results indicate that the culture exposed to high-intensity red light showed the highest biomass yield (2.5 g/L) and lutein productivity (>2.0 mg L-1day-1). However, blue light was found to have a stimulating effect on the synthesis of lutein and other carotenoids (>0.8% dry wt). Furthermore, a direct relationship between lipid accumulation and high light intensity was evidenced. Finally, the highest antioxidant activity was observed with high-intensity white light, these values have no direct relationship with lutein productivity. Therefore, the findings of this study could be utilized to obtain biocompounds of interest by altering certain culture conditions during the large-scale cultivation of MCH-35.

## Intensive production of the harpacticoid copepod Tigriopus californicus in a zero-effluent 'green water' bioreactor

Autor: Alfonso Prado-Cabrero

Sci Rep. 2022 Jan 10;12(1):466. doi: 10.1038/s41598-021-04516-w.

ABSTRACT

Aquaculture is looking for substitutes for fishmeal and fish oil to maintain its continued growth. Zooplankton is the most nutritious option, but its controlled mass production has not yet been achieved. In this context, we have developed a monoalgal 'green water' closed-loop bioreactor with the microalgae Tetraselmis chui that continuously produced the

harpacticoid copepod Tigriopus californicus. During 145 days of operation, the 2.2 m3 bioreactor produced 3.9 kg (wet weight) of Tigriopus with (dry weight) 0.79 ± 0.29% eicosapentaenoic acid (EPA), 0.82 ± 0.26% docosahexaenoic acid (DHA), 1.89 ± 0,60% 3S,3'S-astaxanthin and an essential amino acid index (EAAI) of 97% for juvenile Atlantic salmon. The reactor kept the pH stable over the operation time (pH 8.81 ± 0.40 in the algae phase and pH 8.22 ± 2.96 in the zooplankton phase), while constantly removed nitrate (322.6 mg L-1) and phosphate (20.4 mg L-1) from the water. As a result of the stable pH and nutrient removal, the bioreactor achieved zero effluent discharges. The upscaling of monoalgal, closed-loop 'green water' bioreactors could help standardize zooplankton mass production to supply the aquafeeds industry.

## Interaction between CO2-consuming autotrophy and CO2-producing heterotrophy in non-axenic phototrophic biofilms

Autor: Patrick Ronan

PLoS One. 2021 Jun 15;16(6):e0253224. doi: 10.1371/journal.pone.0253224. eCollection 2021.

ABSTRACT

As the effects of climate change become increasingly evident, the need for effective CO2 management is clear. Microalgae are well-suited for CO2 sequestration, given their ability to rapidly uptake and fix CO2. They also readily assimilate inorganic nutrients and produce a biomass with inherent commercial value, leading to a paradigm in which CO2-sequestration, enhanced wastewater treatment, and biomass generation could be effectively combined. Natural non-axenic phototrophic cultures comprising both autotrophic and heterotrophic fractions are particularly attractive in this endeavour, given their increased robustness and innate O2-CO2 exchange. In this study, the interplay between CO2-consuming autotrophy and CO2-producing heterotrophy in a non-axenic phototrophic biofilm was examined. When the biofilm was cultivated under autotrophic conditions (i.e. no organic carbon), it grew autotrophically and exhibited CO2 uptake. After amending its growth medium with organic carbon (0.25 g/L glucose and 0.28 g/L sodium acetate), the biofilm rapidly toggled from net-autotrophic to net-heterotrophic growth, reaching a CO2 production rate of 60 µmol/h after 31 hours. When the organic carbon sources were provided at a lower concentration (0.125 g/L glucose and 0.14 g/L sodium acetate), the biofilm exhibited distinct, longitudinally discrete regions of heterotrophic and autotrophic metabolism in the proximal and distal halves of the biofilm respectively, within 4 hours of carbon amendment. Interestingly, this upstream and downstream partitioning of heterotrophic and autotrophic metabolism appeared to be reversible, as the position of these regions began to flip once the direction of medium flow (and hence nutrient availability) was reversed. The insight generated here can inform new and important research questions and contribute to efforts aimed at scaling and industrializing algal growth systems, where the ability to understand, predict, and optimize biofilm growth and activity is critical.

## Isolation of Extracellular Vesicles From Microalgae: A Renewable and Scalable Bioprocess

Autor: Angela Paterna

Front Bioeng Biotechnol. 2022 Mar 14;10:836747. doi: 10.3389/fbioe.2022.836747. eCollection 2022.

ABSTRACT

Extracellular vesicles (EVs) play a crucial role as potent signal transducers among cells, with the potential to operate cross-species and cross-kingdom communication. Nanoalgosomes are a subtype of EVs recently identified and isolated from microalgae. Microalgae represent a natural bioresource with the capacity to produce several secondary

metabolites with a broad range of biological activities and commercial applications. The present study highlights the upstream and downstream processes required for the scalable production of nanoalgosomes from cultures of the marine microalgae Tetraselmis chuii. Different technical parameters, protocols, and conditions were assessed to improve EVs isolation by tangential flow filtration (TFF), aiming to enhance sample purity and yield. The optimization of the overall bioprocess was enhanced by quality control checks operated through robust biophysical and biochemical characterizations. Further, we showed the possibility of recycling by TFF microalgae cells post-EVs isolation for multiple EV production cycles. The present results highlight the potential of nanoalgosome production as a scalable, cost-effective bioprocess suitable for diverse scientific and industrial exploitations.

## Macular pigment-enriched oil production from genome-edited microalgae

Autor: Inhwa Song

Microb Cell Fact. 2022 Feb 19;21(1):27. doi: 10.1186/s12934-021-01736-7.

ABSTRACT

BACKGROUND: The photosynthetic microorganism Chlamydomonas reinhardtii has been approved as generally recognized as safe (GRAS) recently, this can excessively produce carotenoid pigments and fatty acids. Zeaxanthin epoxidase (ZEP), which converts zeaxanthin to violaxanthin, and ADP-glucose pyrophosphorylase (AGP). These are key regulating genes for the xanthophyll and starch pathways in C. reinhardtii respectively. In this study, to produce macular pigment-enriched microalgal oil, we attempted to edit the AGP gene as an additional knock-out target in the zep mutant as a parental strain.

RESULTS: Using a sequential CRISPR-Cas9 RNP-mediated knock-out method, we generated double knock-out mutants (dZAs), in which both the ZEP and AGP genes were deleted. In dZA1, lutein (2.93 ± 0.22 mg g-1 DCW: dried cell weight), zeaxanthin (3.12 ± 0.30 mg g-1 DCW), and lipids (450.09 ± 25.48 mg g-1 DCW) were highly accumulated in N-deprivation condition. Optimization of the culture medium and process made it possible to produce pigments and oil via one-step cultivation. This optimization process enabled dZAs to achieve 81% higher oil productivity along with similar macular pigment productivity, than the conventional two-step process. The hexane/isopropanol extraction method was developed for the use of macular pigment-enriched microalgal oil for food. As a result, 196 ± 20.1 mg g-1 DCW of edible microalgal oil containing 8.42 ± 0.92 mg g-1 lutein of oil and 7.69 ± 1.03 mg g-1 zeaxanthin of oil was produced.

CONCLUSION: Our research showed that lipids and pigments are simultaneously induced in the dZA strain. Since dZAs are generated by introducing pre-assembled sgRNA and Cas9-protein into cells, antibiotic resistance genes or selective markers are not inserted into the genome of dZA, which is advantageous for applying dZA mutant to food. Therefore, the enriched macular pigment oil extracted from improved strains (dZAs) can be further applied to various food products and nutraceuticals.

## Metabolite Profiling in Green Microalgae with Varying Degrees of Desiccation Tolerance

Autor: Siegfried Aigner

Microorganisms. 2022 Apr 30;10(5):946. doi: 10.3390/microorganisms10050946.

ABSTRACT

Trebouxiophyceae are microalgae occupying even extreme environments such as polar regions or deserts, terrestrial or aquatic, and can occur free-living or as lichen photobionts. Yet, it is poorly understood how environmental factors shape their metabolism. Here, we report on responses to light and temperature, and metabolic adjustments to desiccation in

Diplosphaera epiphytica, isolated from a lichen, and Edaphochlorella mirabilis, isolated from Tundra soil, assessed via growth and photosynthetic performance parameters. Metabolite profiling was conducted by GC-MS. A meta-analysis together with data from a terrestrial and an aquatic Chlorella vulgaris strain reflected elements of phylogenetic relationship, lifestyle, and relative desiccation tolerance of the four algal strains. For example, compatible solutes associated with desiccation tolerance were up-accumulated in D. epiphytica, but also sugars and sugar alcohols typically produced by lichen photobionts. The aquatic C. vulgaris, the most desiccation-sensitive strain, showed the greatest variation in metabolite accumulation after desiccation and rehydration, whereas the most desiccation-tolerant strain, D. epiphytica, showed the least, suggesting that it has a more efficient constitutive protection from desiccation and/or that desiccation disturbed the metabolic steady-state less than in the other three strains. The authors hope that this study will stimulate more research into desiccation tolerance mechanisms in these under-investigated microorganisms.

## Metagenomics and network analysis elucidating the coordination between fermentative bacteria and microalgae in a novel bacterial-algal coupling reactor (BACR) for mariculture wastewater treatment

Autor: Yedong Gao

Water Res. 2022 Mar 6;215:118256. doi: 10.1016/j.watres.2022.118256. Online ahead of print.

ABSTRACT

To achieve the goal of treating mariculture wastewater economically and efficiently, a novel bacterial-algal coupling reactor (BACR) integrating acidogenic fermentation and microalgae cultivation was firstly investigated for mariculture wastewater treatment. Volatile fatty acids (VFAs) generated in the dark chamber migrated into the photo chamber for microalgal utilization, which alleviated the pH drop and feedback inhibition of the acidogenic fermentation. The maximum dry cell weight (DCW) of microalgae was 1.46 g/L, and pollutants such as chemical oxygen demand (COD), ammonium (NH4+-N) and total phosphorus (TP) in the BACR were effectively removed under the mixotrophic culture condition. Furthermore, bacterial community profiles and functional genes in the BACR and single acidogenic fermentation reactor were identified. Compared with the single acidogenic fermentation reactor, most of the fermentative bacteria (e.g., Ruminococcus, Christensenellaceae R-7 group, Exiguobacterium, Pseudomonas and Levilinea) were enriched by the BACR. From the genetic perspective, the abundances of dominant genes (ackA, acs and atoD) associated with acetic, propionic and butyric acid production were greatly enhanced in the BACR. In the fatty acid biosynthesis pathway (ko00061), three kinds of high-abundance acetyl-CoA carboxylase genes and eight kinds of downstream functional genes were up-regulated in the BACR. Finally, based on co-occurrence network analysis, the coordination between fermentative bacteria and microalgae in the BACR was revealed. This study provided a deep insight into the advantage and potential of the BACR in mariculture wastewater treatment.

## Metal removal capability of two cyanobacterial species in autotrophic and mixotrophic mode of nutrition

Autor: Elham Ghorbani

BMC Microbiol. 2022 Feb 17;22(1):58. doi: 10.1186/s12866-022-02471-8.

ABSTRACT

BACKGROUND: Cyanobacteria are ecologically significant prokaryotes that can be found in heavy metals contaminated environments. As their photosynthetic machinery imposes high demands for metals, homeostasis of these micronutrients has been extensively considered

in cyanobacteria. Recently, most studies have been focused on different habitats using microalgae leads to a remarkable reduction of an array of organic and inorganic nutrients, but what takes place in the extracellular environment when cells are exposed to external supplementation with heavy metals remains largely unknown.

METHODS: Here, extracellular polymeric substances (EPS) production in strains Nostoc sp. N27P72 and Nostoc sp. FB71 was isolated from different habitats and thenthe results were compared and reported.

RESULT: Cultures of both strains, supplemented separately with either glucose, sucrose, lactose, or maltose showed that production of EPS and cell dry weight were boosted by maltose supplementation. The production of EPS (9.1 ± 0.05 μg/ml) and increase in cell dry weight (1.01 ± 0.06 g/l) were comparatively high in Nostoc sp. N27P72 which was isolated from lime stones.The cultures were evaluated for their ability to remove Cu (II), Cr (III), and Ni (II) in culture media with and without maltose. The crude EPS showed metal adsorption capacity assuming the order Ni (II) > Cu (II) > Cr (III) from the metal-binding experiments.Nickel was preferentially biosorbed with a maximal uptake of 188.8 ± 0.14 mg (g cell dry wt) -1 crude EPS. We found that using maltose as a carbon source can increase the production of EPS, protein, and carbohydrates content and it could be a significant reason for the high ability of metal absorbance. FT-IR spectroscopy revealed that the treatment with Ni can change the functional groups and glycoside linkages in both strains. Results of Gas Chromatography-Mass Spectrometry (GC-MS) were used to determine the biochemical composition of Nostoc sp. N27P72, showed that strong Ni (II) removal capability could be associated with the high silicon containing heterocyclic compound and aromatic diacid compounds content.

CONCLUSION: The results of this study indicated that strains Nostoc sp. N27P72 can be a good candidate for the commercial production of EPS and might be utilized in bioremediation field as an alternative to synthetic and abiotic flocculants.

## Microalgae production for nitrogen recovery of high-strength dry anaerobic digestion effluent

Autor: Silvia Greses

Waste Manag. 2022 Feb 15;139:321-329. doi: 10.1016/j.wasman.2021.12.043. Epub 2022 Jan 6.

ABSTRACT

Dry anaerobic digestion (D-AD) generates nitrogen-rich effluents that are normally neglected in the circular bioeconomy. The high turbidity and ammonium content hamper nitrogen recovery from these effluents via biological processes, such as microalgae culture. The goal of this study was to demonstrate microalgae growth viability in high-strength D-AD effluents in order to recover nitrogen (N) as microalgae biomass. According to the experimental factorial design conducted in batch reactors, ammonium was identified as the critical inhibitory compound for microalgae growth while turbidity did not exhibit a significantly negative effect. Instead, turbidity resulted advantageous since it promoted high nitrogen uptake rates and biomass production. The presence of organic turbidity resulted in a positive effect that boosted Chlorella growth in a stream with higher ammonium (350 mg NH4+-N L-1) and turbidity (175 NTU) than the inhibition thresholds reported in the literature, reaching 98.7% of N recovery as microalgae biomass. When microalgae culture was scaled up in a photobioreactor operated in continuous mode, microalgae biomass was effectively produced while recovering 100% of N at a hydraulic retention time of 10 days. By imposing long exposure times and high turbidity, Chlorella adaptation to high-strength D-AD effluent resulted in high N uptake and biomass production. This study demonstrated not only the most influencing factor and the optimal NH4+-N and turbidity combination, but also the viability of using D-AD effluents as culture media for microalgae biomass production.

## Microalgae role in sustainable bioenergy generation as determined by light microscopy

Autor: Moneeza Abbas

Microsc Res Tech. 2022 Jan 3. doi: 10.1002/jemt.24042. Online ahead of print.

ABSTRACT

The main aim of this study was to explore the role of light microscopy in the identification of microalgae as a source of study. Three microalgal species (Nostoc, Anabaena, and Volvox) were identified by light. In this study, different parameters of the oil extraction process from algae biomass were studied. The samples of Nostoc, Anabaena, and Volvox were collected from the freshwater bodies in Lahore, and the samples were identified by light microscopy. Pretreatment of algae was done which includes harvesting, drying, and grinding. The sun drying of sample was done. Solvent extraction was done for the extraction of oil from algal cells. Solvent n-hexane and diethyl ether were used alone as well as in combination. Effects of n-hexane to oil ratio, size of algal biomass, and contact time on the percentage yield of extracted oil were studied and analyzed. It was concluded that maximum amount of oil was extracted from algae by using a greater ratio of solvent to algal biomass, maximum contact time, and smaller algal biomass size. The extracted oil yield was satisfactory, demonstrating the potential of microalgae for biodiesel production. It was discovered that if algal oil is subjected to transesterification, it can be turned into biodiesel, and light microscopy can be used to assess anatomical characteristics. However, more research will be required for transesterification.

## Microalgal Biomass as Feedstock for Bacterial Production of PHA: Advances and Future Prospects

Autor: Florence Hui Ping Tan

Front Bioeng Biotechnol. 2022 May 12;10:879476. doi: 10.3389/fbioe.2022.879476. eCollection 2022.

ABSTRACT

The search for biodegradable plastics has become the focus in combating the global plastic pollution crisis. Polyhydroxyalkanoates (PHAs) are renewable substitutes to petroleum-based plastics with the ability to completely mineralize in soil, compost, and marine environments. The preferred choice of PHA synthesis is from bacteria or archaea. However, microbial production of PHAs faces a major drawback due to high production costs attributed to the high price of organic substrates as compared to synthetic plastics. As such, microalgal biomass presents a low-cost solution as feedstock for PHA synthesis. Photoautotrophic microalgae are ubiquitous in our ecosystem and thrive from utilizing easily accessible light, carbon dioxide and inorganic nutrients. Biomass production from microalgae offers advantages that include high yields, effective carbon dioxide capture, efficient treatment of effluents and the usage of infertile land. Nevertheless, the success of large-scale PHA synthesis using microalgal biomass faces constraints that encompass the entire flow of the microalgal biomass production, i.e., from molecular aspects of the microalgae to cultivation conditions to harvesting and drying microalgal biomass along with the conversion of the biomass into PHA. This review discusses approaches such as optimization of growth conditions, improvement of the microalgal biomass manufacturing technologies as well as the genetic engineering of both microalgae and PHA-producing bacteria with the purpose of refining PHA production from microalgal biomass.

## Nannochloropsis oceanica as a Microalgal Food Intervention in Diet-Induced Metabolic Syndrome in Rats

Autor: Ryan du Preez

Nutrients. 2021 Nov 9;13(11):3991. doi: 10.3390/nu13113991.

ABSTRACT

The microalgal genus Nannochloropsis has broad applicability to produce biofuels, animal feed supplements and other value-added products including proteins, carotenoids and lipids. This study investigated a potential role of N. oceanica in the reversal of metabolic syndrome. Male Wistar rats (n = 48) were divided into four groups in a 16-week protocol. Two groups were fed either corn starch or high-carbohydrate, high-fat diets (C and H, respectively) for the full 16 weeks. The other two groups received C and H diets for eight weeks and then received 5% freeze-dried N. oceanica in these diets for the final eight weeks (CN and HN, respectively) of the protocol. The H diet was high in fructose and sucrose, together with increased saturated and trans fats. H rats developed obesity, hypertension, dyslipidaemia, fatty liver disease and left ventricular fibrosis. N. oceanica increased lean mass in CN and HN rats, possibly due to the increased protein intake, and decreased fat mass in HN rats. Intervention with N. oceanica did not change cardiovascular, liver and metabolic parameters or gut structure. The relative abundance of Oxyphotobacteria in the gut microbiota was increased. N. oceanica may be an effective functional food against metabolic syndrome as a sustainable protein source.

## Optimization of Astaxanthin Recovery in the Downstream Process of Haematococcus pluvialis

Autor: Inga K Koopmann

Foods. 2022 May 6;11(9):1352. doi: 10.3390/foods11091352.

ABSTRACT

Astaxanthin derived from Haematococcus pluvialis is a valuable metabolite applied in a wide range of products. Its extraction depends on a sophisticated series of downstream process steps, including harvesting, disruption, drying, and extraction, of which some are dependent on each other. To determine the processes that yield maximum astaxanthin recovery, bead milling, high-pressure homogenization, and no disruption of H. pluvialis biomass were coupled with spray-drying, vacuum-drying, and freeze-drying in all possible combinations. Eventually, astaxanthin was extracted using supercritical CO2. Optimal conditions for spray-drying were evaluated through the design of experiments and standard least squares regression (feed rate: 5.8 mL/min, spray gas flow: 400 NL/h, inlet temperature: 180 °C). Maximal astaxanthin recoveries were yielded using high-pressure homogenization and lyophilization (85.4%). All combinations of milling or high-pressure homogenization and lyophilization or spray-drying resulted in similar recoveries. Bead milling and spray-drying repeated with a larger spray-dryer resulted in similar astaxanthin recoveries compared with the laboratory scale. Smaller astaxanthin recoveries after the extraction of vacuum-dried biomass were mainly attributed to textural changes. Evaluation of these results in an economic context led to a recommendation for bead milling and spray-drying prior to supercritical CO2 extraction to achieve the maximum astaxanthin recoveries.

## Oxidative stress facilitates infection of the unicellular alga Haematococcus pluvialis by the fungus Paraphysoderma sedebokerense

Autor: Hailong Yan

Biotechnol Biofuels Bioprod. 2022 May 20;15(1):56. doi: 10.1186/s13068-022-02140-y.

ABSTRACT

BACKGROUND: The green microalga Haematococcus pluvialis is used as a cell factory for producing astaxanthin, the high-value carotenoid with multiple biological functions. However, H. pluvialis is prone to the infection by a parasitic fungus Paraphysoderma sedebokerense, which is the most devastating threat to the mass culture of H. pluvialis all over the world. Through dissecting the mechanisms underlying the infection process, effective measures could be developed to mitigate the pathogen threatening for the natural astaxanthin industry. By far, understanding about the interaction between the algal host and fungal pathogen remains very limited.

RESULTS: We observed that there were heat-stable substances with small molecular weight produced during the infection process and enhanced the susceptibility of H. pluvialis cells to the pathogen. The infection ratio increased from 10.2% (for the algal cells treated with the BG11 medium as the control) to 52.9% (for the algal cells treated with supernatant contained such substances) on the second day post-infection, indicating the yet unknown substances in the supernatant stimulated the parasitism process. Systematic approaches including multi-omics, biochemical and imaging analysis were deployed to uncover the identity of the metabolites and the underlying mechanisms. Two metabolites, 3-hydroxyanthranilic acid and hordenine were identified and proved to stimulate the infection via driving oxidative stress to the algal cells. These metabolites generated hydroxyl radicals to disrupt the subcellular components of the algal cells and to make the algal cells more susceptible to the infection. Based on these findings, a biosafe and environment-friendly antioxidant butylated hydroxyanisole (BHA) was selected to inhibit the fungal infection, which completely abolished the infection at 12 ppm. By applying 7 ppm BHA every 2 days to the algal cell culture infected with P. sedebokerense in the 100 L open raceway ponds, the biomass of H. pluvialis reached 0.448 g/L, which was comparable to that of the control (0.473 g/L).

CONCLUSIONS: This study provides for the first time, a framework to dissect the functions of secondary metabolites in the interaction between the unicellular alga H. pluvialis and its fungal parasite, indicating that oxidative degradation is a strategy used for the fungal infest. Eliminating the oxidative burst through adding antioxidant BHA could be an effective measure to reduce parasitic infection in H. pluvialis mass culture.

## Partial Substitution of Fish Oil with Microalgae (Schizochytrium sp.) Can Improve Growth Performance, Nonspecific Immunity and Disease Resistance in Rainbow Trout, Oncorhynchus mykiss

Autor: Seunghan Lee

Animals (Basel). 2022 May 9;12(9):1220. doi: 10.3390/ani12091220.

ABSTRACT

The price of fish oil has reached a historical peak due to a consistent downward production trend, and therefore, the search for sustainable alternative sources has received great attention. This research was conducted to evaluate dietary micro-algae, Schizochytrium sp. (SC) as fish oil (FO) replacer in rainbow trout, Oncorhynchus mykiss. In the first trial, apparent digestibility coefficient (ADC) was 92.4% for dry matter, 91.4% for crude protein, and 94.2% for crude lipid in rainbow trout. In the second trial, six diets were formulated to replace FO at 0% (CON), 20% (T20), 40% (T40), 60% (T60), 80% (T80), and 100% (T100) with SC in the rainbow trout (3.0 ± 0.4 g, mean ± SD) diet. After eight weeks' feeding trial, weight gain (WG), specific growth rate (SGR), and feed efficiency (FE) of fish fed the T20 diet were significantly higher than those of fish fed other diets (p < 0.05). However, there were no significant differences in these parameters among those of fish fed CON, T40, T60, and T80 diets. Lysozyme activity of fish fed the T20 diet was significantly higher than those of fish fed other experimental diets (p < 0.05). After 10 days of disease challenge testing with pathogenic bacteria (Lactococcus garvieae 1 × 108 CFU/mL), the cumulative survival rate of fish fed the T20 diet was significantly higher than those of fish fed the CON, T80, and T100 diets. Therefore, these results suggest dietary microalgae SC is well-digested and could replace up to 80% of fish oil in the diet of rainbow trout without negative effects on growth and immune responses.

## Performance of an immobilized microalgae-based process for wastewater treatment and biomass production: Nutrients removal, lipid induction, microalgae harvesting and dewatering

Autor: Song Cao

Bioresour Technol. 2022 Jul;356:127298. doi: 10.1016/j.biortech.2022.127298. Epub 2022 May 13.

ABSTRACT

Immobilized microalgae are good for wastewater treatment and biomass production. This study investigated treatment efficiency of a continuously operated system employing immobilized microalgae for secondary effluent of wastewater treatment plants, as well as the effectiveness on induction of valuable products, harvesting and dewatering of microalgae biomass. Under semi-continuous operation condition, microalgal dry weight increased 4.75 times within 2 d, associated with the highest removal rate of ammonia and phosphate at 28.95 mg/L·d and 4.83 mg/L·d, respectively. An immobilized microalgae membrane bioreactor (iMBR) was continuously operated for a month. The harvested immobilized microalgae beads were transferred to induction stage to obtain 4.5 times increase of lipid content per cell within 2 d. Immobilized microalgae performed 1.9 cm/s settling velocity and 97% water removal efficiency around 40 °C. A prospective integrated process on resource recovery and carbon neutrality was proposed for wastewater treatment, induction, harvesting and dewatering of immobilized microalgae cells.

## Phycoremediation of milk processing wastewater and lipid-rich biomass production using Chlorella vulgaris under continuous batch system

Autor: Rashmi Verma

Sci Total Environ. 2022 Aug 10;833:155110. doi: 10.1016/j.scitotenv.2022.155110. Epub 2022 Apr 6.

ABSTRACT

This study compiles the results of phycoremediation of milk processing wastewater (MPWW) and production of lipid-rich Chlorella vulgaris biomass using a continuous batch system operated for 12-wks. After a 4-wks interval, a new MPWW was loaded photobioreactor to provide appropriate nutrient supply to algae. Results indicated that MPWW supported the algal growth efficiently and the maximum algal growth was recorded in the ranges of 400.36 to 421.58 mg L-1 during 4-wk's of the cultivation cycle. Average reduction in total nitrogen, TN (45.82-69.18%); nitrate, NO3 (93.32-94.54%); total ammonium nitrogen, TAN (92.94-94.54%); sulphate, SO4-2 (85.13-87.34%); total phosphorus (75.09-78.78%); and biochemical oxygen demands, BOD (89.53-92.40%) was recorded during 12-wks phycoremediation of MPWW. Harvested algal biomass (dry weight basis, DW) exhibited a significant content of total sugar (45.5%) and total lipid (39.7%). The lipid profiling results indicated the presence of palmitic acid (39.9%), oleic acid (21.08%), linoleic acid (13.13%), and other C18 compounds in algal biomass, suggesting the suitability of MPWW for Chlorella vulgaris cultivations. Algal biomass exhibited a high heating value (MJ/Kg of DW) in the range of 17.3 to 25.1, comparable to other lignocellulose biomass to be used for bioenergy purposes. Results of this study indicate that MPWW could be utilized as a valuable medium for Chlorella vulgaris cultivation under a circular economy approach: wastewater treatment and bioenergy feedstock production. The effect of controlled environmental conditions on algal growth behavior and lipid composition in biomass, while using MPWW as a medium, could be investigated in future studies.

## Phytochemical Profiling of Microalgae Euglena tuba and Its Anticancer Activity in Dalton's Lymphoma Cells

Autor: Swati Prabha Gupta

Front Biosci (Landmark Ed). 2022 Apr 1;27(4):120. doi: 10.31083/j.fbl2704120.

ABSTRACT

INTRODUCTION: Natural phytochemicals are considered safe to use as therapeutic agents. There is a growing trend toward exploring anticancer effects of crude algal extracts or their active ingredients. Euglena tuba, a microalga, contains excellent antioxidant potential. However, the anticancer property of E. tuba has not been explored. This study investigates the chemical profiling as well as antitumor property of methanolic extract of E. tuba (ETME) against Dalton's lymphoma (DL) cells.

MATERIALS AND METHODS: E. tuba, procured from northern part of India, was extracted in 70% methanol, dried at room temperature, and stored at -20 ◦C for future use. A freshly prepared aqueous solution of ETME of different concentrations was employed into each experiment. The ETME mediated anti-tumor response in Dalton's lymphoma was evaluated in the inbred populations of BALB/c (H2d) strain of mice of either sex at 8-12 weeks of age. The cytotoxicity of ETME in cancer cells, effects on morphology of cell and nucleus, alteration in the mitochondrial membrane potential, and level of expression of proapoptotic proteins (Bcl-2, cyt C, Bax and p53) were done using known procedures.

RESULTS: The ETME contained high content of total alkaloids (96.02 ± 3.30 mg/100 mg), flavonoids (15.77 ± 2.38 mg/100 mg), carbohydrate (12.71 ± 0.59 mg/100 mg), ascorbic acid (12.48 ± 2.59 mg/100 mg), and phenolics (0.94 ± 0.05 mg/100 mg). Gas chromatography-mass spectrometry (GC-MS) analysis indicated the presence of 23 phytochemicals with known anticancer properties. DL cells treated with ETME exhibited significant and concentration dependent cytotoxicity. Florescent microscopy and flow cytometry of ETME treated DL cells indicated significant repair in cellular morphology and decreased mitochondrial potential, respectively. Western blot analysis displayed up-regulation of proapoptotic proteins (Bax, Cyt-c, p53) and down regulation of anti-apoptotic protein (Bcl2) in DL cells treated with ETME.

CONCLUSIONS: The findings of this study clearly indicated that the anticancer property of ETME was mediated via reduction in mitochondrial potential and induction of apoptotic mechanism. Further studies are warranted to explore the anticancer activities of active ingredients present in this microalga of pharmaceutical importance.

## Pilot-Scale Cultivation of the Snow Alga Chloromonas typhlos in a Photobioreactor

Autor: Floris Schoeters

Front Bioeng Biotechnol. 2022 Jun 9;10:896261. doi: 10.3389/fbioe.2022.896261. eCollection 2022.

ABSTRACT

The most studied and cultivated microalgae have a temperature optimum between 20 and 35°C. This temperature range hampers sustainable microalgae growth in countries with colder periods. To overcome this problem, psychrotolerant microalgae, such as the snow alga Chloromonas typhlos, can be cultivated during these colder periods. However, most of the research work has been carried out in the laboratory. The step between laboratory-scale and large-scale cultivation is difficult, making pilot-scale tests crucial to gather more information. Here, we presented a successful pilot-scale growth test of C. typhlos. Seven batch mode growth periods were compared during two longer growth tests in a photobioreactor of 350 L. We demonstrated the potential of this alga to be cultivated at colder ambient temperatures. The tests were performed during winter and springtime to

compare ambient temperature and sunlight influences. The growth and CO2 usage were continuously monitored to calculate the productivity and CO2 fixation efficiency. A maximum dry weight of 1.082 g L-1 was achieved while a maximum growth rate and maximum daily volumetric and areal productivities of 0.105 d-1, 0.110 g L-1 d-1, and 2.746 g m-2 d-1, respectively, were measured. Future tests to optimize the cultivation of C. typhlos and production of astaxanthin, for example, will be crucial to explore the potential of biomass production of C. typhlos on a commercial scale.

## Pilot-scale outdoor trial of a cyanobacterial consortium at pH 11 in a photobioreactor at high latitude

Autor: Marianne Haines

Bioresour Technol. 2022 Jun;354:127173. doi: 10.1016/j.biortech.2022.127173. Epub 2022 Apr 19.

ABSTRACT

The biomass of microalgae and cyanobacteria yields a variety of products. Outdoor pilot plant trials typically grow a single species at circumneutral pH and provide CO2 by gas sparging. Here a cyanobacterial consortium was grown at high pH (beyond 11) and high dissolved carbonate concentrations (0.5 M) in an outdoor 1,150 L tubular photobioreactor for 130 days in Calgary, Canada. The aim was to assess the productivity and robustness of the consortium. Importantly, the system was designed to enable future integration of air capture of CO2. Productivity was between 3.1 and 5.8 g ash-free dry weight per square metre per day, depending on biomass density and month. 16S rRNA amplicon sequencing showed that cyanobacterium Candidatus "Phormidium alkaliphilum" made up 80% of the consortium. The consortium displayed robust growth and adapted to environmental conditions. Bicarbonate uptake pushed medium pH past 11, demonstrating the ability to achieve CO2 delivery by air capture.

## Protein Enrichment of Wheat Bread with Microalgae: Microchloropsis gaditana, Tetraselmis chui and Chlorella vulgaris

Autor: Waqas Muhammad Qazi

Foods. 2021 Dec 10;10(12):3078. doi: 10.3390/foods10123078.

ABSTRACT

Cell wall disrupted and dried Microchloropsis gaditana (Mg), Tetraselmis chui (Tc) and Chlorella vulgaris (Cv) microalgae biomasses, with or without ethanol pre-treatment, were added to wheat bread at a wheat flour substitution level of 12%, to enrich bread protein by 30%. Baking performance, protein quality and basic sensory properties were assessed. Compared to wheat, Mg, Tc and Cv contain higher amounts of essential amino acids and their incorporation markedly improved protein quality in the bread (DIAAS 57-66 vs. 46%). The incorporation of microalgae reduced dough strength and bread volume and increased crumb firmness. This was most pronounced for Cv and Tc but could be improved by ethanol treatment. Mg gave adequate dough strength, bread volume and crumb structure without ethanol treatment. To obtain bread of acceptable smell, appearance, and colour, ethanol treatment was necessary also for Mg as it markedly reduced the unpleasant smell and intense colour of all algae breads. Ethanol treatment reduced the relative content of lysine, but no other essential amino acids. However, it also had a negative impact on in vitro protein digestibility. Our results show that Mg had the largest potential for protein fortification of bread, but further work is needed to optimize pre-processing and assess consumer acceptance.

## Protein potential of Desmodesmus asymmetricus grown in greenhouse as an alternative food source for aquaculture

Autor: Alberto Oscanoa

World J Microbiol Biotechnol. 2022 Apr 16;38(5):92. doi: 10.1007/s11274-022-03275-8.

ABSTRACT

During ten months, batch culture of Desmodesmus asymmetricus microalgae was carried out under greenhouse conditions. The inoculation ratio was 1:1 (inoculum:treated water). The cultures were maintained for 5 days with natural light and constant aeration mixed with carbon dioxide. The biomass was concentrated by centrifugation and dried by lyophilization; subsequently, total proteins and amino acid concentration were quantified. A relationship between biomass production and seasonal variation was observed, the lowest dry biomass production was recorded in June (38.8 ± 1.0 mg L-1 day-1) and July (43.3 ± 0.1 mg L-1 day-1); while the highest values were greater than 70 mg L-1 day-1 in March. There was a high positive correlation between wet and dry biomass ($r = 0.97$, $p < 0.001$) with a mean conversion of 26%. The mean percentage of protein was 26.1 ± 2.6%, the highest percentage was registered in March (31.03 ± 1.48%) as well as the concentration of amino acids. Regarding amino acids, arginine obtained the highest concentration (4.08 ± 0.43 g 100 g-1), followed by aspartic acid (3.36 ± 0.23 g 100 g-1), while the lowest values were for methionine (0.55 ± 0.21 g 100 g-1), histidine (0.77 ± 0.07 g 100 g-1) and tyrosine (1.01 ± 0.17 g 100 g-1). Finally, according to the essential amino acid index (in fish ≥ 0.90, in crustaceans > 0.80), the biomass of D. asymmetricus has potential as a food supplement for the production of feed in aquaculture.

## Recent progress in flocculation, dewatering, and drying technologies for microalgae utilization: Scalable and low-cost harvesting process development

Autor: Ki Ha Min

Bioresour Technol. 2022 Jan;344(Pt B):126404. doi: 10.1016/j.biortech.2021.126404. Epub 2021 Nov 23.

ABSTRACT

Microalgal research has made significant progress in terms of the high-value-added industrial application of microalgal biomass and its derivatives. However, cost-effective techniques for producing, harvesting, and processing microalgal biomass on a large scale still need to be fully explored in order to optimize their performance and achieve commercial robustness. In particular, technologies for harvesting microalgae are critical in the practical process as they require excessive energy and equipment costs. This review focuses on microalgal flocculation, dewatering, and drying techniques and specifically covers the traditional approaches and recent technological progress in harvesting microalgal biomass. Several aspects, including the characteristics of the target microalgae and the type of final value-added products, must be considered when selecting the appropriate harvesting technique. Furthermore, considerable aspects and possible future directions in flocculation, dewatering, and drying steps are proposed to develop scalable and low-cost microalgal harvesting systems.

## Seasonality of Glacial Snow and Ice Microbial Communities

Autor: Matthias Winkel

Front Microbiol. 2022 May 16;13:876848. doi: 10.3389/fmicb.2022.876848. eCollection 2022.

ABSTRACT

Blooms of microalgae on glaciers and ice sheets are amplifying surface ice melting rates, which are already affected by climate change. Most studies on glacial microorganisms (including snow and glacier ice algae) have so far focused on the spring and summer melt season, leading to a temporal bias, and a knowledge gap in our understanding of the variations in microbial diversity, productivity, and physiology on glacier surfaces year-round. Here, we investigated the microbial communities from Icelandic glacier surface snow and bare ice habitats, with sampling spanning two consecutive years and carried out in both winter and two summer seasons. We evaluated the seasonal differences in microbial community composition using Illumina sequencing of the 16S rRNA, 18S rRNA, and ITS marker genes and correlating them with geochemical signals in the snow and ice. During summer, Chloromonas, Chlainomonas, Raphidonema, and Hydrurus dominated surface snow algal communities, while Ancylonema and Mesotaenium dominated the surface bare ice habitats. In winter, algae could not be detected, and the community composition was dominated by bacteria and fungi. The dominant bacterial taxa found in both winter and summer samples were Bacteriodetes, Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria. The winter bacterial communities showed high similarities to airborne and fresh snow bacteria reported in other studies. This points toward the importance of dry and wet deposition as a wintertime source of microorganisms to the glacier surface. Winter samples were also richer in nutrients than summer samples, except for dissolved organic carbon-which was highest in summer snow and ice samples with blooming microalgae, suggesting that nutrients are accumulated during winter but primarily used by the microbial communities in the summer. Overall, our study shows that glacial snow and ice microbial communities are highly variable on a seasonal basis.

## Separation of Heterotrophic Microalgae Crypthecodinium cohnii by Dielectrophoresis

Autor: Mario Birkholz

Front Bioeng Biotechnol. 2022 May 23;10:855035. doi: 10.3389/fbioe.2022.855035. eCollection 2022.

ABSTRACT

Microalgae constitute an abundant source of poly-unsaturated fatty acids which are applied in various biotechnological fields such as pharmaceuticals and food supplement. Separating microalgae cells with respect to their lipid content would establish a relevant at-line analytical technique. The present study demonstrates an electrical approach for the separation of the lipid-producing microalgae Crypthecodinium cohnii using the effect of dielectrophoresis (DEP) in a microfluidic flow cell. Microalgae were cultivated for 8 days, while cell growth was characterized by optical density, dry cell weight, glucose concentration and lipid content via fluorescence microscopy. The size distribution of cells during cultivation was thoroughly investigated, since the DEP force scales with cell volume, but also depends on lipid content via cell electrophysiological constants. Thus, the challenge was to deconvolute one separation effect from the other, while the electrical cell constants of C. cohnii are not known yet. The DEP-dependent separation was realized by slanted top-bottom electrodes with the flowing cell suspension between them. Turning on the voltage deflected the cells from their initial path as determined by the streaming and thus changed their direction of flow. The separation efficiency of DEP was tested for various electrical field strengths and its performance was determined by quantitative analysis of optical and fluorescence videos. It could be shown for all size groups that the most lipid-containing cells were always subject to DEP separation and that the method is thus not only suitable for process analysis, but also for strain selection of the most productive cell lines.

## Sequential Continuous Mixotrophic and Phototrophic Cultivation Might Be a Cost-Effective Strategy for Astaxanthin Production From the Microalga Haematococcus lacustris

Autor: Mahammed Ilyas Khazi

Front Bioeng Biotechnol. 2021 Oct 5;9:740533. doi: 10.3389/fbioe.2021.740533. eCollection 2021.

ABSTRACT

Although Haematococcus lacustris has been developed for astaxanthin production for decades, the production cost is still high. In order to modify the production processes, we proposed a novel strategy of cultivation, featured by sequential indoor continuous mixotrophic cultivation for the production of green cells followed by outdoor phototrophic induction for astaxanthin accumulation. The continuous mixotrophic cultivation was first optimized indoor, and then the seed culture of mixotrophic cultivation was inoculated into outdoor open raceway ponds for photoinduction. The results showed that mixotrophically grown cultures could efficiently grow without losing their photosynthetic efficiency and yielded higher biomass concentration (0.655 g L-1) and astaxanthin content (2.2% DW), compared to phototrophically grown seed culture controls. This novel strategy might be a promising alternative to the current approaches to advance the production technology of astaxanthin from microalgae.

## Slip slidin' away: Bristle-driven gliding by Tetradesmus deserticola (Chlorophyta) in microfluidic chambers

Autor: Zoe G Cardon

J Phycol. 2022 May 24. doi: 10.1111/jpy.13271. Online ahead of print.

ABSTRACT

Microalgae within the Scenedesmaceae are often distinguished by spines, bristles, and other wall characteristics. We examined the dynamic production and chemical nature of bristles extruded from the poles of Tetradesmus deserticola previously isolated from microbiotic crust. Rapidly growing cells in a liquid growth medium were established in polydimethylsiloxane microfluidic chambers specially designed to maintain aerobic conditions over time within a chamber 6-12 μm deep. This geometry enabled in-focus imaging of single cells over long periods. Differential interference contrast (DIC) imaging revealed that after multiple fission of mother cells, the newly released, lemon-shaped daughter cells began extruding bristles from each pole. In some instances, the bristles became stuck to either the glass floor or polydimethylsiloxane (PDMS) walls of the chamber, and the force by which the new bristle was extruded was sufficient to propel the cells across the field of view at ~1.2 μm · h-1 . Confocal fluorescence and DIC imaging of cells stained with pontamine fast scarlet and calcofluor, and treated with proteinase K, suggested that bristles are proteinaceous and may also host carbohydrate modifications. The polar bristles extruded by this desert-derived T. deserticola may simply be relics of bristles produced by an aquatic ancestor for flotation or predator deterrence. But, their tendency to attach to glass (silicate) and/or PDMS surfaces suggests a potential role in tethering cells in place or binding soil particles. T. deserticola is closely related to T. obliquus, which is of interest for biofuels development; extruded bristles in T. deserticola may offer tethers for industrial use of these stress-tolerant algae.

## Statistical optimization for simultaneous removal of methyl red and production of fatty acid methyl esters using fresh alga Scenedesmus obliquus

Autor: Noura El-Ahmady El-Naggar

Sci Rep. 2022 May 3;12(1):7156. doi: 10.1038/s41598-022-11069-z.

ABSTRACT

Microalgae are a diverse group of microorganisms, the majority of which are photosynthetic in nature. Microalgae have different applications, the most important of which is the biological treatment of wastewater. Microalgae grow in various types of wastewater, such as wastewater polluted by Azo dyes, due to microalgae using wastewater as a culture medium, which contains many nutrients like nitrogen, phosphate, and carbon sources. Microalgae grow in various types of wastewater, such as wastewater polluted by Azo dyes, due to microalgae using wastewater as a culture medium, which contains many nutrients like nitrogen, phosphate, and carbon sources. So, microalgae are used for bioremediation of wastewater due to the efficiency of growing in wastewater and for the high production of lipids followed by trans-esterification to biodiesel. Face-centered central composite design (FCCCD) was used to determine the factors that have the most significant impact on the simultaneous decolorization of methyl red and lipid production by the fresh green alga Scenedesmus obliquus. The predicted results indicated that the alga decolorized 70.15% methyl red and produced 20.91% lipids by using 1 g/L nitrogen, an incubation time of 10 days, a pH of 8, and the concentration of methyl red is 17.65 mg/L. The dry biomasses of S. obliquus were also examined by SEM and FTIR before and after treatment with methyl red. SEM and FTIR showed that the properties of dry S. obliquus were altered after the biosorption of methyl red. According to GC-MS analysis of hexane extracts of S. obliquus, the lipid profile differed before and after methyl red decolorization. The results proved that it is possible to use S. obliquus to remove dyes and produce renewable fuels such as biodiesel. The novelty of this study is that this is the first time in which the effect of nitrogen concentrations in the medium used for algal growth on the removal of dye has been studied.

## Sustainable Bioactive Packaging Based on Thermoplastic Starch and Microalgae

Autor: Anna Martina Tedeschi

Int J Mol Sci. 2021 Dec 24;23(1):178. doi: 10.3390/ijms23010178.

ABSTRACT

This study combines the use of corn starch and Tetradesmus obliquus microalgae for the production of antioxidant starch films as flexible packaging material. Starch was plasticized with glycerol and blended with 1 w% polyallylamine chosen as an agent to modify the film physical properties. The addition of polyallylamine improved film water stability and water vapor transmission rate as well as mechanical stiffness and tenacity. The dried Tetradesmus obliquus microalgae, which showed an EC50 value of 2.8 mg/mg DPPH (2.2-Diphenyl-1-picrylhydrazyl radical), was then used as antioxidant filler. The addition of microalgae provided the films with good antioxidant activity, which increased with microalgae content increasing. To our knowledge, this is the first study reporting the development of sustainable bioactive packaging films composed of almost 100% starch, and follows the European union's goals on plastics strategy concerning the promotion of bio-based, compostable plastics and the setting up of approaches to prevent food waste with a simple plastic packaging.

## Sustainable development of microalgal biotechnology in coastal zone for aquaculture and food

Autor: Xiangning Lu

Sci Total Environ. 2021 Aug 1;780:146369. doi: 10.1016/j.scitotenv.2021.146369. Epub 2021 Mar 10.

ABSTRACT

Region-specific Research and Development (R&D) of microalga-derived product systems are crucial if "biotech's green gold" is to be explored in a rational and economically viable way. Coastal zones, particularly the locations around the equator, are typically considered to be optimum cultivation sites due to stable annual temperature, light, and ready availability of seawater. However, a 'cradle-to-grave' assessment of the development of microalgal biotechnology in these areas, not only under the laboratory conditions, but also in the fields has not yet been demonstrated. In this study, to evaluate the viability of microalga-derived multi-product technology, we showed the development of microalgal biotechnology in coastal zones for aquaculture and food. By creating and screening a (sub)tropical microalgal collection, a Chlorella strain MEM25 with a robust growth in a wide range of salinities, temperatures, and light intensities was identified. Evaluation of the economic viability and performance of different scale cultivation system designs (500 L and 5000 L closed photobioreactors and 60,000 L open race ponds, ORPs) at coastal zones under geographically specific conditions showed the stable and robust characteristics of MEM25 across different production system designs and various spatial and temporal scales. It produces high amounts of proteins and polyunsaturated fatty acids (PUFAs) in various conditions. Feeding experiments reveal the nutritional merits of MEM25 as food additives where PUFAs and essential amino acids are enriched and the algal diet improves consumers' growth. Economic evaluation highlights an appreciable profitability of MEM25 production as human or animal food using ORP systems. Therefore, despite the pros and cons, sound opportunities exist for the development of market-ready multiple-product systems by employing region-specific R&D strategies for microalgal biotechnology.

## Swine wastewater treatment by combined process of iron carbon microelectrolysis-physical adsorption-microalgae cultivation

Autor: Wenjin Zhang

Water Sci Technol. 2022 Feb;85(3):914-924. doi: 10.2166/wst.2021.619.

ABSTRACT

Combined treatments were designed based on iron-carbon micro-electrolysis treatment (ICME), physical adsorption (PA) with zeolite (Z) or vermiculite (V) and microalgae cultivation (MC, Chlorella vulgaris) for removing pollutants from swine wastewater (SW): ICME + MC (IM), ICME + Z + MC (IZM) and ICME + V + MC (IVM). Results showed that the minimum total nitrogen (TN) of 43.66 mg L-1, NH4+-N of 1.33 mg-1 and total phosphorus (TP) of 0.14 mg-1 were obtained by IVM, while the minimum chemical oxygen demand (COD) was 105 mg-1 via IM. During the process of combined treatments, ICME contributed most to the removal of TN (84.52% by IZM), TP (97.78% by IVM and IZM) and COD (62.44% by IVM), and maximum NH4+-N removal (55.64%) was obtained by MC procedure in IM process. Vermiculite performed better than zeolite during all the combined treatments. Besides, the maximum cell dry weight (CDW, 0.74 g-1) of C. vulgaris was obtained by IM on day 13. The results provide an efficient integrated method for swine wastewater treatment.

## Techno-economic assessment of microalgae production, harvesting and drying for food, feed, cosmetics, and agriculture

Autor: Bárbara Vázquez-Romero

Sci Total Environ. 2022 May 5:155742. doi: 10.1016/j.scitotenv.2022.155742. Online ahead of print.

ABSTRACT

The objective of this techno-economic analysis is to define the costs for an industrial microalgae production process, comparing different operation strategies (Nannochloropsis oceanica cultivation during the whole year or cultivation of two species, where Phaeodactylum tricornutum and Tisochrysis lutea alternate), production scales (1 and 10 ha), harvesting technologies (centrifugation or ultrafiltration) and drying methods (freeze-drying or spray drying). This study is based on an industrial scale process established in the south of Portugal. The strategy of cultivating N. oceanica all year round is more attractive from an economic perspective, with production costs of 53.32 €/kg DW and a productivity of 27.61 t/y for a scale of 1 ha, a 49.31% lower cost and two-fold productivity than species alternation culture strategy. These results are for biomass harvested by centrifugation (10.65% biomass cost) and freeze-drying (20.15% biomass cost). These costs could be reduced by 7.03% using a combination of ultrafiltration and spray drying, up to 17.99% if expanded to 10 ha and 10.92% if fertilisers were used instead of commercial nutrient solutions. The study shows potentially competitive costs for functional foods, food, and feed additives, specialised aquaculture products (live feed enrichment) and other high value applications (e.g., cosmetics).

## The first comprehensive study evaluating the ecotoxicity and biodegradability of water-soluble polymers used in personal care products and cosmetics

Autor: Ula Rozman

Ecotoxicol Environ Saf. 2021 Nov 25;228:113016. doi: 10.1016/j.ecoenv.2021.113016. Online ahead of print.

ABSTRACT

Water-soluble polymers (WSPs) are organic materials that have been used for decades in various applications as part of paints, coatings, adhesives, washing agents, pharmaceuticals, personal care products and cosmetics. However, their ecotoxicity, biodegradability, and overall impact on the environment are still unknown. In this study four polyacrylic acid- based WSPs (three in the solid state and one in the liquid state), which are widely used in cosmetic industry, were tested in terms of their ecotoxicity and biodegradability. The ecotoxicity tests were performed using aquatic plant Lemna minor, microalga Pseudokirchneriella subcapitata, crustacean Daphnia magna, bacterium Allivibrio fischeri, and a mixed bacterial culture of activated sludge (with heterotrophic and nitrifying microorganisms tested separately). All four WSPs had low or moderate effects on the tested organisms at several endpoints. However, the liquid WSP had a specific toxic effect on the bioluminescence of Allivibrio fischeri and the oxygen consumption of nitrifying microorganisms - 100 mg/L caused 73% and 88% inhibition, respectively. Therefore, some WSPs capable of inhibiting nitrifying microorganisms could have implications for the nitrification process in wastewater treatment plants and aquatic ecosystems, despite 100 mg/L being a high tested concentration and probably difficult to reach in wastewater. All investigated WSPs were not biodegradable; therefore, their persistence in the environment could be expected.

## Thorsmoerkia curvula gen. et spec. nov. (Trebouxiophyceae, Chlorophyta), a semi-terrestrial microalga from Iceland exhibits high levels of unsaturated fatty acids

Autor: Cecilia Nicoletti

J Appl Phycol. 2021 Dec;33:3671-3682. doi: 10.1007/s10811-021-02577-y. Epub 2021 Sep 9.

ABSTRACT

A terrestrial green alga was isolated at Iceland, and the strain (SAG 2627) was described for its morphology and phylogenetic position and tested for biotechnological capabilities. Cells had a distinctly curved, crescent shape with conical poles and a single parietal chloroplast. Phylogenetic analyses of 18S rDNA and rbcL markers placed the strain into the Trebouxiophyceae (Chlorophyta). The alga turned out to belong to an independent lineage without an obvious sister group within the Trebouxiophyceae. Based on morphological and phylogenetic data, the strain was described as a new genus and species, Thorsmoerkia curvula gen. et sp. nov. Biomass was generated in column reactors and subsequently screened for promising metabolites. Growth was optimized by pH-regulated, episodic CO2 supplement during the logarithmic growth-phase, and half of the biomass was thereafter exposed to nitrogen and phosphate depletion. The biomass yield reached up to 53.5 mg L-1 day-1. Fatty acid (FA) production peaked at 24 mg L-1 day-1 and up to 83% of all FAs were unsaturated. At the end of the log phase, approximately 45% of dry mass were lipids, including eicosapentaenoic acid. Carotenoid production reached up to 2.94 mg L-1 day-1 but it was halted during the stress phase. The N-linked glycans of glycoproteins were assessed to reveal chemotaxonomic patterns. The study demonstrated that new microalgae can be found at Iceland, potentially suitable for applied purposes. The advantage of T. curvula is its robustness and that significant amounts of lipids are already accumulated during log phase, making a subsequent stress exposure dispensable.

## Total Phenolic Levels, In Vitro Antioxidant Properties, and Fatty Acid Profile of Two Microalgae, Tetraselmis marina Strain IMA043 and Naviculoid Diatom Strain IMA053, Isolated from the North Adriatic Sea

Autor: Riccardo Trentin

Mar Drugs. 2022 Mar 12;20(3):207. doi: 10.3390/md20030207.

ABSTRACT

This work studied the potential biotechnological applications of a naviculoid diatom (IMA053) and a green microalga (Tetraselmis marina IMA043) isolated from the North Adriatic Sea. Water, methanol, and dichloromethane (DCM) extracts were prepared from microalgae biomass and evaluated for total phenolic content (TPC) and in vitro antioxidant properties. Biomass was profiled for fatty acid methyl esters (FAME) composition. The DCM extracts had the highest levels of total phenolics, with values of 40.58 and 86.14 mg GAE/g dry weight (DW in IMA053 and IMA043, respectively). The DCM extracts had a higher radical scavenging activity (RSA) than the water and methanol ones, especially those from IMA043, with RSAs of 99.65% toward 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) at 10 mg/mL, and of 103.43% against 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 5 mg/mL. The DCM extract of IMA053 displayed relevant copper chelating properties (67.48% at 10 mg/mL), while the highest iron chelating activity was observed in the water extract of the same species (92.05% at 10 mg/mL). Both strains presented a high proportion of saturated (SFA) and monounsaturated (MUFA) fatty acids. The results suggested that these microalgae could be further explored as sources of natural antioxidants for the pharmaceutical and food industry and as feedstock for biofuel production.

## Transcription Factors From Haematococcus pluvialis Involved in the Regulation of Astaxanthin Biosynthesis Under High Light-Sodium Acetate Stress

Autor: Chaogang Wang

Front Bioeng Biotechnol. 2021 Oct 25;9:650178. doi: 10.3389/fbioe.2021.650178. eCollection 2021.

ABSTRACT

The microalgae Haematococcus pluvialis attracts attention for its ability to accumulate astaxanthin up to its 4% dry weight under stress conditions, such as high light, salt stress, and nitrogen starvation. Previous researches indicated that the regulation of astaxanthin synthesis might happen at the transcriptional level. However, the transcription regulatory mechanism of astaxanthin synthesis is still unknown in H. pluvialis. Lacking studies on transcription factors (TFs) further hindered from discovering this mechanism. Hence, the transcriptome analysis of H. pluvialis under the high light-sodium acetate stress for 1.5 h was performed in this study, aiming to discover TFs and the regulation on astaxanthin synthesis. In total, 83,869 unigenes were obtained and annotated based on seven databases, including NR, NT, Kyoto Encyclopedia of Genes and Genomes Orthology, SwissProt, Pfam, Eukaryotic Orthologous Groups, and Gene Ontology. Moreover, 476 TFs belonging to 52 families were annotated by blasting against the PlantTFDB database. By comparing with the control group, 4,367 differentially expressed genes composing of 2,050 upregulated unigenes and 2,317 downregulated unigenes were identified. Most of them were involved in metabolic process, catalytic activity, single-organism process, single-organism cellular process, and single-organism metabolic process. Among them, 28 upregulated TFs and 41 downregulated TFs belonging to 27 TF families were found. The transcription analysis showed that TFs had different transcription modules responding to the high light and sodium acetate stress. Interestingly, six TFs belonging to MYB, MYB_related, NF-YC, Nin-like, and C3H families were found to be involved in the transcription regulation of 27 astaxanthin synthesis-related genes according to the regulatory network. Moreover, these TFs might affect astaxanthin synthesis by directly regulating CrtO, showing that CrtO was the hub gene in astaxanthin synthesis. The present study provided new insight into a global view of TFs and their correlations to astaxanthin synthesis in H. pluvialis.

## Transcriptome analysis of lipid metabolism in response to cerium stress in the oleaginous microalga Nannochloropsis oculata

Autor: Di Wu

Sci Total Environ. 2022 Sep 10;838(Pt 3):156420. doi: 10.1016/j.scitotenv.2022.156420. Epub 2022 Jun 1.

ABSTRACT

Nannochloropsis oculata can accumulate large amounts of lipids under rare earth element (REE) conditions. However, the lipid accumulation mechanism responsible for REE stress has not been elucidated. In this study, the effects of cerium (the most abundant REE) on the growth and lipid accumulation of N. oculata were investigated. The de novo transcriptome data of N. oculata under cerium conditions were subsequently collected and analyzed. The results showed that N. oculata exhibited good cerium-resistance ability, showed slightly decrease in biomass but significantly increase in lipid content (55.8 % dry cell weight) under 6.0 mg/L cerium condition. Meanwhile, about 83.4 % cerium was biological fixated. Through transcriptome analysis, we found that the inhibited photosynthesis and carbon fixation pathways coupled with the stress-sensitive expression of ribosome biogenesis genes acclimatized the cells to REE stress. The active glycolysis pathway accelerated carbon flux to pyruvate and acetyl-CoA, and the upregulation of glycerol kinase and phosphatidate cytidylyltransferase genes further induced lipid accumulation. In addition, cerium downregulated the acyl-CoA oxidase and triacylglycerol lipase genes, which inhibited the degradation of lipids. Therefore, different responses to

cerium demonstrate how N. oculata cells adapt to REE stress, and this knowledge may be used to extend our understanding of triacylglycerol (TAG) and the synthesis of other important metabolites.

## Transcriptome and Metabolome Profiling of a Novel Isolate Chlorella sorokiniana G32 (Chlorophyta) Displaying Enhanced Starch Accumulation at High Growth Rate Under Mixotrophic Condition

Autor: Qingling Zhu

Front Microbiol. 2022 Jan 6;12:760307. doi: 10.3389/fmicb.2021.760307. eCollection 2021.

ABSTRACT

Chlorella sorokiniana is one of the most productive microalgal species with a high potential for the production of biofuels and other high value-added molecules. Many studies have focused on its capability of mixotrophic growth using reduced organic carbon and growth pattern shift between autotrophic and mixotrophic conditions. In this study, we investigated growth patterns of a novel isolate, C. sorokiniana G32, under mixotrophic growth conditions supplemented with a low level (1.25 g L-1) and a high level (5 g L-1) of glucose. Physiological, transcriptomic (i.e., RNA-seq), and metabolomic (i.e., LC-MS/MS) methods were used. We showed that peak growth based on OD680nm absorbance is ~4-fold higher with high glucose vs. low glucose supplementation. Photosynthetic efficiency (Fv/Fm) in G32 mixotrophic cultures with high or low glucose supplementation remains identical to that of G32 phototrophic growth. We also found that the conversion rate between absorbance-based cell density and cell dry weight with high glucose supplementation was lower than with low glucose. This suggests that more cell biomass is produced under high glucose treatment than with low glucose. The result was confirmed via sucrose density gradient centrifugation. It is likely that accumulation of high concentration of starch may account for this effect. Transcriptomic analysis of G32 cultures (i.e., via RNA-seq) in response to reciprocal change of glucose levels reveals that expression of a subset of differentially expressed genes (DEGs) is correlated with the amount of glucose supplementation. These DEGs are designated as glucose-specific responsive (GSR) genes. GSR genes are enriched for a number of energy metabolic pathways. Together with metabolomics data (i.e., LC-MS/MS), we show that under high-level supplementation, glucose is preferentially oxidized through an oxidative pentose phosphate pathway. Collectively, our results indicate the mechanism of regulation of glucose assimilation and energy metabolism in G32 under mixotrophic conditions with different levels of glucose supplementation revealed by transcriptomic and metabolomic analyses. We propose that C. sorokiniana G32 has the potential for the production of high value-added molecules.

## Trophic transfer of copper decreases the condition index in Crassostrea gigas spat in concomitance with a change in the microalgal fatty acid profile and enhanced oyster energy demand

Autor: F Akcha

Sci Total Environ. 2022 Feb 15;824:153841. doi: 10.1016/j.scitotenv.2022.153841. Online ahead of print.

ABSTRACT

Due to new usages and sources, copper (Cu) concentrations are increasing in the Arcachon Basin, an important shellfish production area in France. In the present paper, the trophic transfer of Cu was studied between a microalga, Tetraselmis suecica, and Crassostrea gigas (Pacific oyster) spat. An experimental approach was developed to assess Cu

exposure, transfer and toxicity on both phytoplankton and spat. Exposure of microalgal cultures to Cu for 7-8 days (3.1 ± 0.1, 15.7 ± 0.2 and 50.4 ± 1.0 µg Cu·L-1 for the control, Cu15 and Cu50 conditions, respectively) led to concentrations in microalgae (28.3 ± 0.9 and 110.7 ± 11.9 mg Cu·kg dry weight-1 for Cu15 and Cu50, respectively) close to those measured in the field. Despite Cu accumulation, the physiology of the microalgae remained poorly affected. Exposed cultures could only be discriminated from controls by a higher relative content in intracellular reactive oxygen species, and a lower relative content in lipids together with a reduced metabolic activity. By contrast, the fatty acid profile of microalgae was modified, with a particularly relevant lower content of the essential polyunsaturated fatty acid 22:6n-3 (docosahexaenoic acid [DHA]). Following 21 days of spat feeding with Cu15 and Cu50 microalgal cultures, trophic transfer of Cu was observed with a high initial Cu concentration in spat tissues. No effect was observed on oxidative stress endpoints. Cu exposure was responsible for a decrease in the spat condition index, an outcome that could be related to an insufficient DHA supply and extra energy demand as suggested by the overexpression of genes involved in energy metabolism, ATP synthesis and glycogen catabolism.

## Ultrasound for microalgal cell disruption and product extraction: A review

Autor: Ying Liu

Ultrason Sonochem. 2022 Jun;87:106054. doi: 10.1016/j.ultsonch.2022.106054. Epub 2022 Jun 1.

ABSTRACT

Microalgae are a promising feedstock for the production of biofuels, nutraceuticals, pharmaceuticals and cosmetics, due to their superior capability of converting solar energy and CO2 into lipids, proteins, and other valuable bioactive compounds. To facilitate the release of these important biomolecules from microalgae, effective cell disruption is usually necessary, where the use of ultrasound has gained tremendous interests as an alternative to traditional methods. This review not only summarizes the mechanisms of and operation parameters affecting cell disruption, but also takes an insight into measuring techniques, synergistic integration with other disruption methods, and challenges of ultrasonication for microalgal biorefining. Optimal conditions including ultrasonic frequency, intensity, and duration, and liquid viscosity and sonochemical reactor are the key factors for maximizing the disruption and extraction efficiency. A combination of ultrasound with other disruption methods such as ozonation, microwave, homogenization, enzymatic lysis, and solvents facilitates cell disruption and release of target compounds, thus provides powerful solutions to commercial scale-up of ultrasound extraction for microalgal biorefining. It is concluded that ultrasonication is a sustainable "green" process, but more research and work are needed to upscale this process without sacrificing performance or consuming more energy.

## Ultrasound-Assisted Extraction of Nannochloropsis oculata with Ethanol and Betaine: 1,2-Propanediol Eutectic Solvent for Antioxidant Pigment-Rich Extracts Retaining Nutritious the Residual Biomass

Autor: Maria D Gkioni

Antioxidants (Basel). 2022 May 31;11(6):1103. doi: 10.3390/antiox11061103.

ABSTRACT

The aim of this study was the development of an efficient "green" extraction method of Nannochloropsis oculata to produce antioxidant extracts and nutritious residual biomass. Twenty-one extraction methods were evaluated by measuring the reactivity with the Folin-Ciocalteu reagent: ultrasonication or maceration at different temperatures with different organic solvents, extraction at different pH values, enzyme-assisted extraction, encapsulation with β-cyclodextrin, and the use of natural deep eutectic solvents. Ultrasound-assisted extraction with ethanol or betaine: 1,2-propanediol in a molar ratio of

2:5 (BP) had optimal extractive capacity. Both extracts were evaluated with antioxidant assays and the ethanol extract exhibited significantly higher (at least twofold) values. The determination of carotenoids by LC-MS and HPLC-DAD revealed the dominance of violaxanthin and antheraxanthin and their fourfold higher concentrations in the ethanol extract. The 1H-NMR characterization of the ethanol extract confirmed the results of the colorimetric and chromatographic assays. The microalgal biomass was characterized before and after the extraction in terms of humidity, ash, carbohydrates, proteins, chlorophyll-a, carotenoids, and lipids; the identity and content of the latter were determined with gas chromatography. BP caused a smaller depletion of the lipids from the biomass compared to ethanol, but proteins, carbohydrates, and ash were at a higher content in the biomass obtained after ethanol extraction, whereas the biomass was dry and easy to handle. Although further optimization may take place for the scale-up of those procedures, our study paves the way for a green strategy for the valorization of microalgae in cosmetics without generating waste, since the remaining biomass can be used for aquafeed.

## Unexpected acceleration of Ultrasonic-Assisted iodide dosimetry in the catalytic presence of ionic liquids

Autor: Sooridarsan Krishnan

Ultrason Sonochem. 2021 Jun;74:105576. doi: 10.1016/j.ultsonch.2021.105576. Epub 2021 Apr 25.

ABSTRACT

This study investigates the potential of using small amounts of ionic liquids (IL) to enhance ultrasound-assisted extraction of lipids content from green microalgae. Three imidazolium-based ILs (butyl, octyl and dodecyl), each of them with two anions (bromide and acetate) were tested as additives. Viscosity and surface tension of the ILs aqueous mixtures were analyzed to determine the influence of ILs' anions and alkyl chain length, whereas KI dosimetry experiments were used as an indicator of radicals formation. A key finding suggests that the small addition of ILs improves the ultrasonication either by enhancing the viscosity and reducing the water surface tension, leading to a more powerful acoustic cavitation process or by increasing HO° production likely to oxidize the microalgae cells membranes, and consequently disrupting them on a more efficient manner. KI dosimetry also revealed that long ILs alkyl chain is detrimental. This experimental observation is confirmed thus strengthened as the yield of extracted lipids from green microalgae has shown an incremental trend when the IL concentration also increased. These hypotheses are currently under investigation to spot detailed impact of ILs on cavitation process.

## Use of Microalgae Biomass for Fortification of Food Products from Grain

Autor: Julia Bazarnova

Foods. 2021 Dec 5;10(12):3018. doi: 10.3390/foods10123018.

ABSTRACT

This article describes the use of Chlorella sorokiniana biomass additives in pasta recipes to enrich the product with biologically active phytonutrients, as well as to achieve the desired color range without the use of synthetic dyes. Samples of dry biomass were obtained by the cultivation of microalgae C. sorokiniana (strain), its quality indicators and nutritional value were determined for use as a food additive. A method of using dry biomass of microalgae C. sorokiniana as a phytoadditive to replace 5% of flour mixture for effective enrichment of pasta with biologically active phytonutrients was proposed. The choice of the optimal amount of addition of microalgae biomass was proved since it turned out that the replacement of flour should be no more than 5% due to the distinct fish flavor of the final product. The present study was conducted to evaluate the effect of adding dry biomass of Chlorella microalgae on total protein, lipid, chlorophyll, and carotenoid content. Substitution of 5% of pasta flour led to an increase in the content of proteins and lipids to

15.7 ± 0.50% and 4.1 ± 0.06%, respectively. Meanwhile, the addition of microalgae Chlorella to pasta has helped to increase the content of polyunsaturated fatty acids, chlorophyll, and carotenoids which are necessary for the prevention of foodborne diseases. The aim of this study is to develop pasta recipe with additives of microalgae biomass C. sorokiniana and study their quality indicators.

## Using chlorella vulgaris for nutrient removal from hydroponic wastewater: experimental investigation and economic assessment

Autor: Y I D Yousif

Water Sci Technol. 2022 Jun;85(11):3240-3258. doi: 10.2166/wst.2022.157.

ABSTRACT

The study evaluated the use of Chlorella vulgaris for bioremediating hydroponic wastewater and producing biomass under different cultivation modes and to explore the economic implications of microalgal biofuels. Total nitrogen (TN) removal efficiency was 98.5% in mixotrophic conditions and 96% in heterotrophic conditions, and total phosphorus (TP) was completely removed (>99%) in both cultivation conditions. TN removal was higher for that which was cultivated under the mixotrophic mode of cultivation. The maximum biomass production (1.26 g/L) and biomass productivity (0.1108 g/L/day) were also reported for mixotrophic conditions. Lipid content was slightly higher for that which was cultivated under heterotrophic conditions: 33 wt% on an ash-free dry weight (AFDW) basis. The highest lipid production was obtained under mixotrophic growth (0.341 g/L). Higher net profit was obtained for both mixotrophic and heterotrophic cultivations: 30.6 million $/year for a plant capacity of 3.29 × 104 tone/year and 30.12 million $/year for a plant capacity of 3.17 × 104 tone/year respectively. Sensitivity analysis showed that biodiesel and nutritious supplements from soluble protein have the greatest impact on the process economics regarding mixotrophic cultivation, while biodiesel and feeds from insoluble protein have the largest effect on the process economics regarding heterotrophic and autotrophic cultivations.

## Waste-to-wealth application of wastewater treatment algae-derived hydrochar for Pb(II) adsorption

Autor: Jiuling Yu

MethodsX. 2021 Feb 7;8:101263. doi: 10.1016/j.mex.2021.101263. eCollection 2021.

ABSTRACT

Hydrochar, as an energy-lean solid waste, is generated from an advanced biofuel conversion technique hydrothermal liquefaction (HTL) and always leads to environmental pollution without appropriate disposal. In this study, HTL-derived hydrochar is recycled and prepared as adsorbent used for Pb(Ⅱ) removal from wastewater. As the original porous structure of hydrochar is masked by oily volatiles remained after HTL, two types of oil-removal pretreatment (Soxhlet extraction and CO2 activation) are explored. The result shows that CO2 activation significantly enhances the adsorption capacity of Pb(Ⅱ), and the maximum adsorption capacity is 12.88 mg g-1, as evaluated using Langmuir adsorption model. Further, apart from oily volatiles, most inorganic compounds derived from wastewater-grown algae is enriched in hydrochar, causing a smaller surface area of hydrochar. An ash-removal alkali treatment following CO2 activation is introduced to dramatically increase the adsorption capacity to 25.00 mg g-1 with an extremely low Pb(II) equilibrium concentration of 5.1×10-4 mg L-1, which is much lower than the maximum level of Pb concentration in drinking water (set by World Health Organization). This work introduces an approach to reuse HTL-hydrochar as an inexpensive adsorbent in Pb-contaminated water treatment, which not only provides another possible renewable adsorbent candidate applied in the field of lead adsorption, but also finds an alternative route to reduce solid waste effluent from HTL process.

# Water-plasma-enhanced and phase-separation-assisted extraction of microalgal lipid for biodiesel production

Autor: Dong Liang

Bioresour Technol. 2022 Jun;354:127198. doi: 10.1016/j.biortech.2022.127198. Epub 2022 Apr 20.

ABSTRACT

Traditional methods for lipid extraction from microalgal biomass usually involve harsh reaction conditions or the use of contaminant reagents, which lead to enormous energy consumption and wastage. Hence, a novel strategy was presented, which combined water-plasma and three-phase partitioning (TPP) techniques. Benefiting from its unique advantages such as rapid and low cost, water-plasma strategy can disrupt microalgal cell wall and can thus greatly affect lipid extraction. As a result, assisted with the TPP method, excellent performance lipid recovery (74.34%) was obtained at 200 W in 10 min. The performance was superior to that achieved through cell disruption via water-plasma pretreatment. Importantly, the whole process of lipid extraction prevented the drying of microalgal biomass, contributing to reduced energy consumption in large-scale biodiesel production. Moreover, the high fatty acids content suggested that the extracted lipids are great potential candidate for biodiesel production.

## PATENTES

### Bacteria-algae coupling integrated equipment and oil production and decontamination method

Autor: LI LIXIN; FU RAN

The invention belongs to the technical field of bacterium-alga decontamination and oil production, and discloses bacterium-alga coupling integrated equipment and an oil production decontamination method, and the method comprises the following steps: installing a reactor, and preparing a suspension and nanoparticles; adding water into bacteria-algae coupling integrated equipment, inoculating a proper amount of suspension, concentrating algae liquid, adding a certain amount of nanoparticles, an active oxygen inducer and a carbon sequestration enhancer, and culturing bacteria-algae coupling particles; adding the alkaline desorption liquid into the reactor, and carrying out bacterium-alga regeneration based on the bacterium-alga coupling particles; meanwhile, mature bacteria and algae are taken out and dried, dry thalli are obtained, and the grease yield is calculated based on the dry thalli. According to the method, the sewage can be degraded to reach the standard and discharged, meanwhile, the yield of mature microalgae extracted bio-oil is greatly increased, and a high-added-value product is produced. The aim of emission reduction is achieved in the intermediate treatment process, and the operation cost is reduced due to the output of bio-oil.

### CONVERSION OF ALGAE TO BIOMETHANE

TECHNICAL FIELDTHE INVENTION PERTAINS TO HIGH YIELD CULTIVATION OF MICROALGAE BIOMASS IN OUTDOOR RACEWAYS THROUGH CAREFUL CONTROL OF ALL GROWTH PARAMETERS AND THE ADJUSTMENT OF THE ALGAE INTERNAL COMPOSITION IN ORDER TO OBTAIN A SUPERIOR VOLUME OF METHANE PER UNIT OF BIOMASS THROUGH ANAEROBIC DIGESTION.A SELECTED SPECIES OF MICROALGAE IS SEEDED IN COVERED RACEWAYS AND GROWN UNDER STRICTLY CONTROLLED CONDITIONS OF NUTRIENT AND MICRONUTRIENT ADDITION, CO2 ADDITION, ARTIFICIAL LIGHT OF SPECIFIC SPECTRUM FOR THE ALGAL SPECIES AND THE APPLICATION OF A PULSED MAGNETIC FIELD. THE RACEWAY SOLUTION IS CONSTANTLY AGITATED TO ENSURE SUNLIGHT REACHES ALL OF THE MICROALGAE IN THE RACEWAY. THE OBJECTIVE OF CONTROLLING ALL GROWTH PARAMETERS IS TO MAXIMIZE THE CREATION OF BIOMASS BY PROVIDING, IN A TIMELY FASHION, THE ELEMENTS NEEDED BY THE MICROALGAE AS IDENTIFIED IN LABORATORY STUDIES.THE PULSED MAGNETIC FIELD IMPROVES THE GROWTH RATE OF THE ALGAE WHILE MAINTAINING A GOOD CELL DENSITY ( STIMULATION OF GROWTH AND METABOLIC CASCADES) BY CONTROLLING BIOCHEMICAL PATHWAYS. IN ADDITION THE PHOTOTROPIC CELLULAR COMMUNICATION IS ENHANCED LEADING TO A HIGHER CONTENT OF LIGHT HARVESTING PRIMARY AND ACCESSORY PIGMENTS. THIS RESULTS IN AN IMPORTANT INCREASE IN LIPID CONTENT OF THE ALGAE BIOMASS WHICH IN TURN YIELDS A HIGHER PRODUCTION OF METHANE COMPARED TO THE CARBOHYDRATE AND PROTEIN CONTENTS IN THE BIOMASS.THE PROCESS RECYCLES THE MAXIMUM QUANTITY OF WATER POSSIBLE AS WELL AS CAPTURING ALL THE CO2 REMOVED FROM THE SCRUBBING OF THE BIOGAS AND RECYCLING IT TO THE RACEWAYS TO PROMOTE ALGAE GROWTH.

## Composite seaweed fertilizer and preparation method thereof

Autor: FANG QINJING; FU ZHIYONG (2)

The invention discloses a composite seaweed fertilizer and a preparation method thereof. The composite seaweed fertilizer comprises raw materials and auxiliary materials. The fertilizer is prepared from the following raw material components in parts by weight: 7-9 parts of seaweed, 5-7 parts of algae residues, 6-8 parts of microalgae residues, 4-6 parts of ascophyllum nodosum, 1-3 parts of Ecklonia kurome, 4-6 parts of kelp, 3-5 parts of gulfweed, 2-4 parts of Chewangzao, 2-4 parts of desert algae, 2-4 parts of Rhodomelaceae, 3-5 parts of fucus, 1-3 parts of enteromorpha, 1-3 parts of Ulva lactuca, 3-5 parts of Kappaphycus, 3-5 parts of brown algae, 2-4 parts of citrus peel residues, 1-3 parts of zeolite, 4-6 parts of shells and 4-6 parts of urea. According to the composite seaweed fertilizer and the preparation method thereof, compared with an organic fertilizer, thec omposite seaweed fertilizer use algae as main raw material, the cost can be effectively reduced, the environment optimization effect can be achieved, the manufacturing method changes a traditional evaporation and concentration process, energy consumption is reduced, and through the processes of drying, cooling, reacting, fermenting and the like, the composite seaweed fertilizer has the advantages that various nutrients in the algae are fully used, the cost is conveniently reduced, the application range of the seaweed fertilizer is widened, the environment is effectively protected and the like.

## Household garbage incineration resource comprehensive utilization algae production method

Autor: LI HAOMING; DOU MINGYUAN (3)

The invention discloses a household garbage incineration resource comprehensive utilization algae production method. The method comprises the following process steps: (1) pre-sorting household garbage, feeding pre-sorted tailings into a storage material preheating container for preheating, drying and dehydrating; (2) incinerating the preheated household garbage in an incinerator; (3) making flue gas and waste heat generated by incineration enter a lime kiln to burn quick lime; (4) introducing the flue gas from the lime kiln into an auxiliary combustion chamber for sufficient combustion, then introducing the flue gas into a water washing tower for dust removal, and cooling to 30 DEG C at the same time; (5) introducing the flue gas exhausted from the water washing tower into an algae culturepond for culturing microalgae, and carrying out circulating heat exchange on hot water and water for the algae culture pond through a heat exchanger; and (6) safely discharging residual flue gas. According to the method, flue gas and waste heat generated by incineration of the household garbage are used for firing lime, purification of the flue gas can be achieved, CO2 can also be generated to beused for cultivating microalgae rich in grease, and economic benefits and social benefits are created while the emission standard is met.

## INTEGRATED BIOGAS-MICROALGAE PROCESS AND PLANT FOR CARRYING OUT THE SAME

Autor: VELEA SANDA [RO]; GALAN ANA-MARIA [RO] (2)

The invention relates to an integrated process for producing biogas from microalgae and to a plant for carrying out said process. According to the invention, the process has the following stages: 1. preparing the organic substrate for the initial feeding and for the subsequent periodical feedings of the digestor with the same waste as in the initial substrate to which the spent microalgal biomass is added, as well as the amount of phytocatalysts extracted therefrom and subjected to wet grinding up to a particle size <5mm; 2. charging the digestor and starting the recycling and the process of monitoring the anaerobic co-digestion; 3. periodically discharging an amount of raw digestate and recovering the liquid digestate and the solid digestate, after the operations of settling, filtration, bleaching performed with the electrocoagulation/settling equipment, for recovery

thereof as nutrient medium for the culture of microalgae and further processing by thermocatalytic pyrolysis; 4. culturing the microalgae in microalgae-microorganisms consortia systems, in autotrophic/mixotrophic conditions, in open basins, with day/night illumination cycle, using Zarrouk nutrient medium; 5. periodical harvesting of an amount of 10% microalgal biomass by electrocoagulation, flocculation, settling, while obtaining phytocatalysts; 6. monitoring the biogas volume and composition, compressing and storing the same; and 7. conditioning of the solid digestate for its thermocatalytic processing. The claimed plant consists of a digestor (DA), a basin for algae culture (BCA), a vessel for preparation of the nutrient solution (V1), a digestate collecting vessel (V2), a separate liquid digestate collecting vessel (V3), a filtered water collecting vessel (V4), a harvesting vessel (VR), a mobile homogenizer for the substrate (Om), electrocoagulation-flocculation equipment (ECF), a central pump (PC), centrifugal pumps (P1, P2 and P3), a compressor (CP), an expansion valve (RE), a safety valve (SS) and an electromagnetic valve (VE)

## Low-consumption green system and method for recovering carbon, nitrogen and phosphorus resources in sewage

Autor: ZHANG JIAN; XIE HUIJUN (5)

The invention relates to the technical field of environmental protection and sewage treatment, in particular to a low-consumption green system and method for recovering carbon, nitrogen and phosphorus resources in sewage. The system comprises a micro-aerobic biological adsorption tank, an inclined plate sedimentation tank and a microalgae culture tank which are connected in sequence, wherein the runway ponds in the microalgae culture tank are connected in a common-wall manner, an aeration device at the bottom of the microalgae culture tank adopts a micro-nano aeration pipe, and based on the system, a micro-aerobic biological adsorption-microalgae culture coupling process method is adopted. According to the method, under the conditions of low energy consumption, no addition of chemical agents and no secondary environmental pollution, the carbon, nitrogen and phosphorus resources in the sewage can be recovered by recovering the residual sludge and the microalgae biomass.

## Method for co-producing gas-oil-feed by coupling anaerobic digestion of marine product processing waste with microalgae culture

Autor: ZHANG WANLI; WANG XUE (3)

The invention relates to a method for co-producing gas-oil-feed by coupling anaerobic digestion of marine product processing waste with microalgae culture. The method comprises the following steps of (1) pulping the marine product processing waste; (2) carrying out anaerobic fermentation reaction to generate biogas, biogas slurry and biogas residue; (3) biogas residue drying treatment; (4) desulfurizing and drying the biogas, using one part for combustion power generation, and separating CO2 and biological methane from the other part; (5) diluting the biogas slurry with water, adding trichloroisocyanuric acid for sterilization treatment, then performing aeration treatment, and adding a sodium carbonate solution to adjust the pH value to 6.8-8; (6) feeding into an oil-rich microalgae culture system to generate oil-rich microalgae; and (7) squeezing out bio-oil from the oil-rich microalgae, and returning the residual algae residue to an anaerobic fermentation reactor. According to the method disclosed by the invention, marine waste treatment, biogas purification, microalgae culture and microalgae-prepared high-quality bio-oil can be integrated, so that efficient operation and energy self-sufficiency are realized by controlling process conditions.

## Method for increasing content of aldol phospholipid in microalgae and RNA interference fragment

Autor: HU HANHUA; PAN YUFANG

The invention relates to a method for increasing the content of acetal phospholipid in microalgae. The method comprises the following steps: down-regulating expression of phospholipid: diglyceride acyltransferase in the microalgae; the invention also relates to an RNA interference fragment capable of improving the acetal phospholipid content in nannochloropsis oculata, the RNA interference fragment comprises a pair of reverse repeat regions, and the sequence of the reverse repeat regions is homologous with the mRNA sequence of the nPDAT gene; the invention also relates to an expression vectorof the RNA interference fragment. The nannochloropsis oculata is subjected to genetic modification, expression of the nPDAT gene in the nannochloropsis oculata is reduced, the acetal phospholipid content in the nannochloropsis oculata is greatly increased to about 150 nmol/g from about 50 nmol/g dry weight, culture conditions are optimized in the culture process, the acetal phospholipid content isfurther increased by about 360 nmol/g, and a possible way is provided for commercialized production of acetal phospholipids by using microalgae.

## Method for preparing high-value feedstuff for aquatic products by using microalgae and artemia salina

Autor: SUN ZHENG; WANG CAN (2)

The invention discloses a method for preparing high-value feedstuff for aquatic products by using microalgae and artemia salina. The high-value feedstuff for the aquatic products is prepared through culturing nauplii of artemia with the microalgae, and then, carrying out collecting, sterilizing, freeze-drying and crushing, wherein the microalgae comprise at least one of dunaliella salina, chlorella salina, isochrysis galbana, nannochloropsis oculata and platymonas subcordiformis. According to the method disclosed by the invention, the specific high-value microalgae serve as a unique food supply of the nauplii of artemia, a one-step food cycle culture mode is employed, the microalgae have multiple nutrients and a high value, can be used for promoting and improving the growth, development and immunity of the artemia salina and have the characteristics of high artemia salina survival rate, high nutritive value, environmental-friendliness, safety and simple culture process; and due to aftertreatment, the appearance, color and luster of the artemia salina can be completely reserved to the maximum, the damage to the nutrients is less, nutritive values for the feedstuff for the aquatic products are reserved relatively greatly, and the prepared feedstuff for the aquatic products is rich in nutrition and relatively low in cost, does not pollute culture water and has an application prospect.

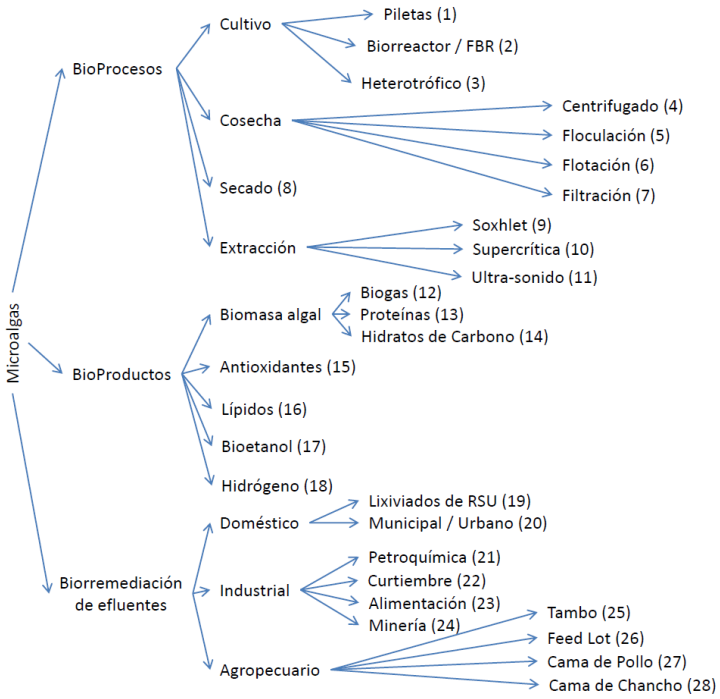
## Multifunctional microalgae protein milk and preparation method thereof

Autor: LI LINPIN; ZHANG YONG (1)

The invention discloses multifunctional microalgae protein milk and a preparation method thereof. The multifunctional microalgae protein milk and the preparation method thereof comprise the following steps of: firstly, taking a raw material, i.e., microalgae, cleaning, drying and smashing the microalgae, adding purified water into the microalgae, in addition, carrying out heating and reflux extraction for 2-4 hours, and then, carrying out filtering to obtain microalgae extraction filtrating liquid; enabling the microalgae extraction filtrating liquid to stand, and carrying out cooling until the temperature is 35 DEG C or below, and obtaining microalgae filtrating liquid; and carrying out enzymolysis processing, regulating the pH value of the obtained microalgae filtrating liquid by acid to 3.5-5, then, adding cellulase, carrying out first-time enzymolysis at the enzymolysis temperature of 45-55 DEG C for enzymolysis time of 50-80min, and obtaining an enzymolysis solution A. By use of the multifunctional microalgae protein milk and the preparation method thereof, the enzymolysis is carried out for two times in a preparation process, the microalgae can be fully degraded so as to be favorable for digestion and absorption of the human body, and meanwhile, the beverage has a smooth taste, a high nutrient value and a good utilization prospect and is simple in operation.

## Árbol de categorías

Español




Inglés