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Microalgas

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A chromosome-level genome assembly for the astaxanthin-producing microalga *Haematococcus pluvialis*

Autor: Chao Bian

Sci Data. 2023 Aug 3;10(1):511. doi: 10.1038/s41597-023-02427-1.

ABSTRACT

The green microalga *Haematococcus pluvialis* can synthesize high amounts of astaxanthin, which is a valuable antioxidant that has been utilized in human health, cosmetics, and aquaculture. To illustrate detailed molecular clues to astaxanthin yield, we performed PacBio HiFi along with Hi-C sequencing to construct an improved chromosome-level haplotypic genome assembly with 32 chromosomes and a genome size of 316.0 Mb. Its scaffold N50 (942.6 kb) and contig N50 (304.8 kb) have been upgraded remarkably from our previous genome draft, and a total of 32,416 protein-coding genes were predicted. We also established a high-evidence phylogenetic tree from seven representative algae species, with the main aim to calculate their divergence times and identify expanded/contracted gene families. We also characterized genome-wide localizations on chromosomes of some important genes such as five BKTs (encoding beta-carotene ketolases) that are putatively involved in astaxanthin production. In summary, we reported the first chromosome-scale map of *H. pluvialis*, which provides a valuable genetic resource for in-depth biomedical investigations on this momentous green alga and commercial astaxanthin bioproduction.

A comprehensive dataset on the effects of *Nannochloropsis* sp. inclusion diets on water quality and oxidative stress of guppy (*Poecilia reticulata*)

Autor: Razia Sultana

Data Brief. 2022 Dec 13;46:108820. doi: 10.1016/j.dib.2022.108820. eCollection 2023 Feb.

ABSTRACT

This experiment was designed to collect the data on antioxidants content of guppy fed with *Nannochloropsis* sp. inclusion diets through partial replacement of fishmeal in feed and the effect of microalgal diet on water quality parameter of the culture system. Triplicate groups of fifteen uniform sized guppy fries were kept in each rectangular glass tank (20 L) maintaining the male and female ratio to 1:2. Different experimental diets containing *Nannochloropsis* sp. (0%-control; 5%-N5, 10%-N10 and 15%-N15) and commercial feed (CMF) were fed to the fishes, two times a day at 5% of their body weight for 100 consecutive days. Water quality parameters were analyzed and recorded throughout the trial period. Both physical and chemical parameters of the culture tanks were measured during the trial period. At the end of experiment, random sampling was done for growth parameter assessment and further laboratory analysis. Oxidative stress (hydrogen peroxide and lipid peroxidation) was analyzed with the carcass sample. In this study, antioxidants content of guppy showed a significant difference among the treatment. Also, improved water quality parameters were found in the treatment tanks where guppy were fed with microalgae formulated feeds. In conclusion, results from this study indicate that selected marine microalga can increase the antioxidant properties of fish that would help in production of more hardy culture species for commercial aqua farming as well as help to maintain water quality parameters of the culture system which is now become a great problem.

A waste-to-wealth initiative exploiting the potential of *Anabaena variabilis* for designing an integrated biorefinery

Autor: Dipanwita Deb

Sci Rep. 2022 Jun 8;12(1):9478. doi: 10.1038/s41598-022-13244-8.

ABSTRACT



The current research work was an innovative approach providing dual advantages of waste bioremediation and an effective biorefinery. The study attempted to exploit wastewater like aqua discharge and solid wastes like poultry litter/cow dung for cyanobacterial cultivation. Aqua discharge appended with 7.5 g L⁻¹ poultry litter turned out as the best combination generating 46% higher carbohydrate yield than BG-11 control. *A. variabilis* cultivation in this waste-utilized medium also revealed its excellent bioremediation ability. While 100% removal was observed for nitrite, nitrate, and orthophosphate, a respective 74% and 81% reduction was noted for ammonium and total organic carbon. Chemical and biological oxygen demands were also reduced by 90%. This work was also novel in developing a sequential design for the production of bioethanol and co-products like exopolysaccharides, sodium copper chlorophyllin, C-phycoerythrin, and poly- β -hydroxybutyrate from the same cyanobacterial biomass. The developed biorefinery implementing the waste-utilized medium was one of its kind, enabling biomass valorization of 61%. Therefore, the present study would provide a leading-edge for tackling the high production costs that limit the practical viability of biorefinery projects. The recyclability of the bioremediated wastewater would not only curtail freshwater usage, the waste disposal concerns would also be mitigated to a great extent.

Antioxidant, Antiviral, and Anti-Inflammatory Activities of Lutein-Enriched Extract of *Tetraselmis* Species

Autor: Eun-A Kim

Mar Drugs. 2023 Jun 21;21(7):369. doi: 10.3390/md21070369.

ABSTRACT

Microalgae are proposed to have powerful applications for human health in the pharmaceutical and food industries. *Tetraselmis* species (sp.), which are green microalgae, were identified as a source of broad-spectrum health-promoting biological activities. However, the bioactivity of these species has not been elucidated. We aimed to confirm the antioxidant, antiviral, and anti-inflammatory effects of *Tetraselmis* sp. extract (TEE). TEE showed 2,2-diphenyl-1-picryl-hydrazyl-hydrate radical and hydrogen peroxide scavenging activities and reduced plaque formation in Vero E6 cells infected with vaccinia virus. TEE treatment also significantly inhibited nitric oxide (NO) production and improved cell viability in lipopolysaccharide (LPS)-induced RAW264.7 cells. These anti-inflammatory effects were further analyzed in LPS-induced RAW 264.7 cells and the zebrafish model. Further, TEE reduced induced NO synthase expression and proinflammatory cytokine release, including tumor necrosis factor- α , interleukin-6, and interleukin-1 β , through MAPKs and NF- κ B-dependent mechanisms. Further analysis revealed that TEE increased the survival rate and reduced cell death and NO production in an LPS-stimulated zebrafish model. Further, high-performance liquid chromatography revealed a strong presence of the carotenoid lutein in TEE. Overall, the results suggest that lutein-enriched TEE may be a potent antioxidant, antiviral, and anti-inflammatory agent that could be sustainably utilized in industrial applications.

Application of Pulsed Electric Fields and High-Pressure Homogenization in Biorefinery Cascade of *C. vulgaris* Microalgae

Autor: Daniele Carullo

Foods. 2022 Feb 5;11(3):471. doi: 10.3390/foods11030471.

ABSTRACT

In this study, a cascaded cell disintegration process, based on pulsed electric fields (PEF - 20 kV/cm, 100 kJ/kgSUSP.) and high-pressure homogenization (HPH - 150 MPa, 5 passes) was designed for the efficient and selective release of intracellular compounds (water-soluble proteins, carbohydrates, and lipids) from *C. vulgaris* suspensions during extraction in water (25 °C, 1 h) and ethyl acetate (25 °C, 3 h). Recovery yields of target compounds from cascaded treatments (PEF + HPH) were compared with those observed when applying PEF and HPH treatments individually. Particle size distribution and scanning electron microscopy analyses showed that PEF treatment alone did not induce any measurable effect on cell shape/structure, whereas HPH caused complete cell fragmentation and debris formation, with an undifferentiated release of intracellular matter. Spectra measurements



demonstrated that, in comparison with HPH alone, cascaded treatments increased the selectivity of extraction and improved the yields of carbohydrates and lipids, while higher yields of water-soluble proteins were measured for HPH alone. This work, therefore, demonstrates the feasibility of sequentially applying PEF and HPH treatments in the biorefinery of microalgae, projecting a beneficial impact in terms of process economics due to the potential reduction of the energy requirements for separation/purification stages.

Application of a novel biological-nanoparticle pretreatment to *Oscillatoria acuminata* biomass and coculture dark fermentation for improving hydrogen production

Autor: Mostafa El-Sheekh

Microb Cell Fact. 2023 Feb 22;22(1):34. doi: 10.1186/s12934-023-02036-y.

ABSTRACT

BACKGROUND: Energy is the basis and assurance for a world's stable development; however, as traditional non-renewable energy sources deplete, the development and study of renewable clean energy have emerged. Using microalgae as a carbon source for anaerobic bacteria to generate biohydrogen is a clean energy generation system that both local and global peers see as promising.

RESULTS: *Klebsiella pneumonia*, *Enterobacter cloacae*, and their coculture were used to synthesize biohydrogen using *Oscillatoria acuminata* biomass via dark fermentation. The total carbohydrate content in *O. acuminata* was 237.39 mg/L. To enhance the content of fermentable reducing sugars, thermochemical, biological, and biological with magnesium zinc ferrite nanoparticles (Mg-Zn Fe₂O₄-NPs) pretreatments were applied. Crude hydrolytic enzymes extracted from *Trichoderma harzianum* of biological pretreatment were enhanced by Mg-Zn Fe₂O₄-NPs and significantly increased reducing sugars (230.48 mg/g) four times than thermochemical pretreatment (45.34 mg/g). *K. pneumonia* demonstrated a greater accumulated hydrogen level (1022 mLH₂/L) than *E. cloacae* (813 mLH₂/L), while their coculture showed superior results (1520 mLH₂/L) and shortened the production time to 48 h instead of 72 h in single culture pretreatments. Biological pretreatment + Mg-Zn Fe₂O₄ NPs using coculture significantly stimulated hydrogen yield (3254 mLH₂/L), hydrogen efficiency)216.9 mL H₂/g reducing sugar(and hydrogen production rate (67.7 mL/L/h) to the maximum among all pretreatments.

CONCLUSION: These results confirm the effectiveness of biological treatments + Mg-Zn Fe₂O₄-NPs and coculture dark fermentation in upregulating biohydrogen production.

Application of microalgae *Scenedesmus acuminatus* enhances water quality in rice-crayfish culture

Autor: Danni Yuan

Front Bioeng Biotechnol. 2023 May 4;11:1143622. doi: 10.3389/fbioe.2023.1143622. eCollection 2023.

ABSTRACT

Improper management of aquatic environments substantially restricts the development of the aquaculture industry. The industrialisation of the crayfish *Procambarus clarkii*, for example, is currently being limited by poor water quality. Research suggests that microalgal biotechnology has a great potential for water quality regulation. However, the ecological effects of microalgal applications on aquatic communities in aquaculture systems remain largely unknown. In the present study, 5 L *Scenedesmus acuminatus* GT-2 culture (biomass 120 g L⁻¹) was added to an approximately 1,000 m² rice-crayfish culture to examine the response of aquatic ecosystems to microalgal application. The total nitrogen content decreased significantly as a result of microalgal addition. Moreover, the microalgal addition changed the bacterial community structure directionally and produced more nitrate reducing and aerobic bacteria. The effect of microalgal addition on plankton community structure was not obvious, except for a significant difference in *Spirogyra* growth which was inhibited by 81.0% under microalgal addition. Furthermore, the network of microorganisms in culture systems with the added microalga had higher interconnectivity and was more complex, which indicating microalgal application enhance the stability of aquaculture systems. The application of microalgae was found to have the greatest effect on the 6th day of the experiment, as supported by both environmental and



Assessing the potential of *Chlorella* sp. phycoremediation liquid digestates from brewery wastes mixture integrated with bioproduct production

Autor: Sen Wang

Front Bioeng Biotechnol. 2023 Jun 14;11:1199472. doi: 10.3389/fbioe.2023.1199472. eCollection 2023.

ABSTRACT

Digestates from different anaerobic digesters are promising substrates for microalgal culture, leading to effective wastewater treatment and the production of microalgal biomass. However, further detailed research is needed before they can be used on a large scale. The aims of this study were to investigate the culture of *Chlorella* sp. in DigestateM from anaerobic fermentation of brewer's grains and brewery wastewater (BWW) and to explore the potential use of the biomass produced under different experimental conditions, including diverse cultivation modes and dilution ratios. Cultivation in DigestateM initiated from 10% (v/v) loading, with 20% BWW, obtained maximum biomass production, reaching 1.36 g L⁻¹ that was 0.27g L⁻¹ higher than 1.09 g L⁻¹ of BG11. In terms of DigestateM remediation, the maximum removal of ammonia nitrogen (NH₄⁺-N), chemical oxygen demand, total nitrogen, and total phosphorus reached 98.20%, 89.98%, 86.98%, and 71.86%, respectively. The maximum lipid, carbohydrate, and protein contents were 41.60%, 32.44%, and 27.72%, respectively. The growth of *Chlorella* sp. may be inhibited when the Y(II)-Fv/Fm ratio is less than 0.4.

Assessment of Antioxidant and Anticancer Activities of Microgreen Alga *Chlorella vulgaris* and Its Blend with Different Vitamins

Autor: Ragaa A Hamouda

Molecules. 2022 Feb 28;27(5):1602. doi: 10.3390/molecules27051602.

ABSTRACT

There is a very vital antioxidant extracted from microgreen alga. *Chlorella vulgaris* has major advantages and requires high yield worldwide. Some microalgae require vitamins for their growth promotion. This study was held to determine the impact of different vitamins including Thiamine (B1), Riboflavin (B2), Pyridoxine (B6), and Ascorbic acid (c) at concentrations of 0.02, 0.04, 0.06, and 0.08 mg/L of each. Each vitamin was added to the BG11 growth medium to determine the effect on growth, total carbohydrate, total protein, pigments content, antioxidant activities of *Chlorella vulgaris*. Moreover, antitumor effects of methanol extract of *C. vulgaris* without and with the supplement of thiamine against Human prostate cancer (PC-3), Hepatocellular carcinoma (HEPG-2), Colorectal carcinoma (HCT-116) and Epithelioid Carcinoma (Hela) was estimated in vitro. *C. vulgaris* supplemented with various vitamins showed a significant increase in biomass, pigment content, total protein, and total carbohydrates in comparison to the control. Thiamine was the best vitamin influencing as an antioxidant. *C. vulgaris* supplemented with thiamine had high antitumor effects in vitro. So, it's necessary to add vitamins to BG11 media for enhancement of the growth and metabolites.

Assessment of Eicosapentaenoic Acid (EPA) Production from Filamentous Microalga *Tribonema aequale*: From Laboratory to Pilot-Scale Study

Autor: Jijian Long

Mar Drugs. 2022 May 24;20(6):343. doi: 10.3390/md20060343.

ABSTRACT

It has long been explored to use EPA-rich unicellular microalgae as a fish oil alternative for production of the high-value omega-3 fatty acid eicosapentaenoic acid (EPA, 20:5, n-3). However, none of the efforts have ever reached commercial success. This study reported a filamentous yellow-green microalga *Tribonema aequale* that possesses the ability to grow



rapidly and synthesizing significant amounts of EPA. A series of studies were conducted in a glass column photobioreactor under laboratory culture conditions and in pilot-scale open raceway ponds outdoors. The emphasis was placed on the specific nutrient requirements and the key operational parameters in raceway ponds such as culture depth and mixing regimes. When optimized, *T. aequale* cells contained 2.9% of EPA (w/w) and reached a very high biomass concentration of 9.8 g L⁻¹ in the glass column photobioreactor. The cellular EPA content was increased further to 3.5% and the areal biomass and EPA productivities of 16.2 g m⁻² d⁻¹ and 542.5 mg m⁻² d⁻¹, respectively, were obtained from the outdoor pilot-scale open raceway ponds, which were the record high figures reported thus far from microalgae-based EPA production. It was also observed that *T. aequale* was highly resistant to microbial contamination and easy for harvesting and dewatering, which provide two additional competitive advantages of this filamentous microalga over the unicellular counterparts for potential commercial production of EPA and other derived co-products.

Associated bacterial microbiome responds opportunistic once algal host *Scenedesmus vacuolatus* is attacked by endoparasite *Amoebophilidium protococcarum*

Autor: Anna-Lena Hoeger

Sci Rep. 2022 Aug 1;12(1):13187. doi: 10.1038/s41598-022-17114-1.

ABSTRACT

The interactions of microalgae and their associated microbiomes have come to the fore of applied phylogenetic research in recent years. However, the functional mechanisms of microalgal interactions remain largely unknown. Here, we examine functional protein patterns of the microalgae *Scenedesmus vacuolatus* and its associated bacterial community during algal infection by the endoparasite *Amoebophilidium protococcarum*. We performed metaproteomics analyses of non-infected (NI) and aphelid-infected (AI) *S. vacuolatus* cultures to investigate underlying functional and physiological changes under infectious conditions. We observed an increase in bacterial protein abundance as well as a severe shift of bacterial functional patterns throughout aphelid-infection in comparison to NI treatment. Most of the bacterial proteins (about 55%) upregulated in AI were linked to metabolism and transport of amino acids, lipids, coenzymes, nucleotides and carbohydrates and to energy production. Several proteins associated with pathogenic bacterial-plant interactions showed higher protein abundance levels in AI treatment. These functional shifts indicate that associated bacteria involved in commensalistic or mutualistic interactions in NI switch to opportunistic lifestyles and facilitate pathogenic or saprotrophic traits in AI treatment. In summary, the native bacterial microbiome adapted its metabolism to algal host die off and is able to metabolize nutrients from injured cells or decompose dead cellular material.

Asterarcys quadricellulare (Chlorophyceae) protects H9c2 cardiomyoblasts from H₂O₂-induced oxidative stress

Autor: Imen Saadaoui

Mol Cell Biochem. 2023 Sep;478(9):1915-1925. doi: 10.1007/s11010-022-04626-7. Epub 2022 Dec 30.

ABSTRACT

Oxidative stress has recently been identified as an important mediator of cardiovascular diseases. The need to find efficient antioxidant molecules is essential in the disease's prevention. Therefore, the present study aimed to evaluate the potential of microalgae bioactive in protecting H9c2 cardiomyoblasts from H₂O₂-induced oxidative stress. Four microalgal species were investigated for their antioxidant capacity. A qualitative assessment of oxidative stress in H9c2 cardiomyoblasts stained with DCFH-DA, treated with the highly active microalgae extracts, was performed. The protein expression of total caspase-3 was also examined to investigate whether the extract protects H9c2 cardiomyoblasts from H₂O₂-induced apoptosis. High antioxidant activity was observed for the hexanoic extracts after 10 days of cultivation. *Asterarcys quadricellulare* exhibited the highest antioxidant capacity of 110.59 ± 1.75 mg TE g⁻¹ dry weight and was tested against H9c2 cardiomyoblasts, which were initially subjected to H₂O₂-induced oxidative



stress. This hexanoic extract protected against H₂O₂ induced oxidative stress with a similar scavenging capacity as N-Acetylcysteine. Furthermore, total caspase-3 was increased following treatment with the hexanoic extract, suggesting that *A. quadricellulare* also had anti-apoptotic properties. The outcome of our study highlighted the possible use of the local *A. quadricellulare* strain QUCCCM10 as a natural, safe, and efficient antioxidant to prevent cardiovascular diseases.

Biopolymer production from biomass produced by Nordic microalgae grown in wastewater

Autor: Sanjeet Mehariya
Bioresour Technol. 2023 May;376:128901. doi: 10.1016/j.biortech.2023.128901. Epub 2023 Mar 16.

ABSTRACT

Biomass from four different Nordic microalgal species, grown in BG-11 medium or synthetic wastewater (SWW), was explored as inexpensive carbohydrate-rich feedstock for polyhydroxybutyrate (PHB) production via microbial fermentation. Thermochemical pre-treatment (acid treatment followed by autoclavation) with 2% hydrochloric acid or 1% sulphuric acid (v/v) was used to maximize sugar yield prior to fermentation. Pre-treatment resulted in ~5-fold higher sugar yield compared to the control. The sugar-rich hydrolysate was used as carbon source for the PHB-producing extremophilic bacterium *Halomonas halophila*. Maximal PHB production was achieved with hydrolysate of *Chlorococcum* sp. (MC-1) grown on BG-11 medium (0.27 ± 0.05 g PHB/ g DW), followed by hydrolysate derived from *Desmodesmus* sp. (RUC-2) grown on SWW (0.24 ± 0.05 g PHB/ g DW). Nordic microalgal biomass grown on wastewater therefore can be used as cheap feedstock for sustainable bioplastic production. This research highlights the potential of Nordic microalgae to develop a biobased economy.

Characterization of Silver Nanoparticle Systems from Microalgae Acclimated to Different CO₂ Atmospheres

Autor: Shirley Mora-Godínez
ACS Omega. 2023 Jun 5;8(24):21969-21982. doi: 10.1021/acsomega.3c01914. eCollection 2023 Jun 20.

ABSTRACT

Green synthesis of metallic nanoparticles using microalgae exposed to high CO₂ atmospheres has not been studied in detail; this is of relevance in biological CO₂ mitigation systems where considerable biomass is produced. In this study, we further characterized the potential of an environmental isolate *Desmodesmus abundans* acclimated to low and high CO₂ atmospheres [low carbon acclimation (LCA) and high carbon acclimation (HCA) strains, respectively] as a platform for silver nanoparticle (AgNP) synthesis. As previously characterized, cell pellets at pH 11 were selected from the biological components tested of the different microalgae, which included the culture collection strain *Spirulina platensis*. AgNP characterization showed superior performance of strain HCA components as preserving the supernatant resulted in synthesis in all pH conditions. Size distribution analysis evidenced strain HCA cell pellet platform (pH 11) as the most homogeneous AgNP population (14.9 ± 6.4 nm diameter, -32.7 ± 5.3 mV) followed by *S. platensis* (18.3 ± 7.5 nm, -33.9 ± 2.4 mV). In contrast, strain LCA presented a broader population where the size was above 100 nm (127.8 ± 14.8 nm, -26.7 ± 2.4 mV). Fourier-transform infrared and Raman spectroscopies showed that the reducing power of microalgae might be attributed to functional groups in the cell pellet from proteins, carbohydrates, and fatty acids and, in the supernatant, from amino acids, monosaccharides, disaccharides, and polysaccharides. Microalgae AgNPs exhibited similar antimicrobial properties in the agar diffusion test against *Escherichia coli*. However, they were not effective against Gram (+) *Lactobacillus plantarum*. It is suggested that a high CO₂ atmosphere potentiates components in the *D. abundans* strain HCA for nanotechnology applications.



Characterization of cellular toxicity induced by sub-lethal inorganic mercury in the marine microalgae *Chlorococcum dorsiventrale* isolated from a metal-polluted coastal site

Autor: Jihen Thabet

Chemosphere. 2023 Oct;338:139391. doi: 10.1016/j.chemosphere.2023.139391. Epub 2023 Jul 4.

ABSTRACT

Mercury (Hg) is a global pollutant that affects numerous marine aquatic ecosystems. We isolated *Chlorococcum dorsiventrale* Ch-UB5 microalga from coastal areas of Tunisia suffering from metal pollution and analyzed its tolerance to Hg. This strain accumulated substantial amounts of Hg and was able to remove up to 95% of added metal after 24 and 72 h in axenic cultures. Mercury led to lesser biomass growth, higher cell aggregation, significant inhibition of photochemical activity, and appearance of oxidative stress and altered redox enzymatic activities, with proliferation of starch granules and neutral lipids vesicles. Such changes matched the biomolecular profile observed using Fourier Transformed Infrared spectroscopy, with remarkable spectral changes corresponding to lipids, proteins and carbohydrates. *C. dorsiventrale* accumulated the chloroplastic heat shock protein HSP70B and the autophagy-related ATG8 protein, probably to counteract the toxic effects of Hg. However, long-term treatments (72 h) usually resulted in poorer physiological and metabolic responses, associated with acute stress. *C. dorsiventrale* has potential use for Hg phycoremediation in marine ecosystems, with the ability to accumulating energetic reserves that could be used for biofuel production, supporting the notion of using of *C. dorsiventrale* for sustainable green chemistry in parallel to metal removal.

Characterization, antioxidant and anticoagulant properties of exopolysaccharide from marine microalgae

Autor: Zahra Mousavian

AMB Express. 2022 Mar 3;12(1):27. doi: 10.1186/s13568-022-01365-2.

ABSTRACT

The sulfated exopolysaccharide extracted from marine microalgae attracted considerable attention from both the nutraceutical and pharmaceutical industries. In the present study biomass of five marine microalgae were screened to find strains with high capacity for the production of sulfated exopolysaccharides. The anticoagulant and antioxidant activities of extracted sulfated polysaccharides were evaluated using activated partial thromboplastin time (aPTT), prothrombin time (PT), DPPH and ABTS assays, respectively. The sulfated polysaccharides extracted from *Picochlorum* sp. showed a strong DPPH scavenging effect with 85% antioxidant activity. The sulfated polysaccharides of *Chlorella sorokiniana*, *Chlorella* sp. (L2) and *Chlorella* sp. (D1) scavenged more than 90% of the ABTS radicals. However, the sulfated polysaccharide extracted from *Chlorella sorokiniana*, and *Chlorella* sp. (N4) showed anticoagulant properties. The dual anticoagulant-antioxidant activities in *Chlorella sorokiniana* could be explained by the combination of various factors including sulfate content and their binding site, monosaccharide residue and glycoside bond which are involved in the polysaccharide's bioactivity.

Chlamydomonas-*Methylobacterium oryzae* cooperation leads to increased biomass, nitrogen removal and hydrogen production

Autor: María Jesús Torres

Bioresour Technol. 2022 May;352:127088. doi: 10.1016/j.biortech.2022.127088. Epub 2022 Mar 29.

ABSTRACT

In the context of algal wastewater bioremediation, this study has identified a novel consortium formed by the bacterium *Methylobacterium oryzae* and the microalga *Chlamydomonas reinhardtii* that greatly increase biomass generation (1.22 g L⁻¹·d⁻¹),



inorganic nitrogen removal (>99%), and hydrogen production (33 mL-L⁻¹) when incubated in media containing ethanol and methanol. The key metabolic aspect of this relationship relied on the bacterial oxidation of ethanol to acetate, which supported heterotrophic algal growth. However, in the bacterial monocultures the acetate accumulation inhibited bacterial growth. Moreover, in the absence of methanol, ethanol was an unsuitable carbon source and its incomplete oxidation to acetaldehyde had a toxic effect on both the alga and the bacterium. In cocultures, both alcohols were used as carbon sources by the bacteria, the inhibitory effects were overcome and both microorganisms mutually benefited. Potential biotechnological applications in wastewater treatment, biomass generation and hydrogen production are discussed.

Chloroplast engineering of the green microalgae *Chlamydomonas reinhardtii* for the production of HAA, the lipid moiety of rhamnolipid biosurfactants

Autor: Bernat Miró-Vinyals

N Biotechnol. 2023 Sep 25;76:1-12. doi: 10.1016/j.nbt.2023.03.005. Epub 2023 Mar 31.

ABSTRACT

Hydroxyalkanoyloxyalkanoates (HAA) are lipidic surfactants with a number of potential applications, but more remarkably, they are the biosynthetic precursors of rhamnolipids (RL), which are preferred biosurfactants thanks to their excellent physicochemical properties, biological activities, and environmental biodegradability. Because the natural highest producer of RLs is the pathogenic bacterium *Pseudomonas aeruginosa*, important efforts have been dedicated to transfer production to heterologous non-pathogenic microorganisms. Unicellular photosynthetic microalgae are emerging as important hosts for sustainable industrial biotechnology due to their ability to transform CO₂ efficiently into biomass and bioproducts of interest. Here, we have explored the potential of the eukaryotic green microalgae *Chlamydomonas reinhardtii* as a chassis to produce RLs. Chloroplast genome engineering allowed the stable functional expression of the gene encoding RhIA acyltransferase from *P. aeruginosa*, an enzyme catalyzing the condensation of two 3-hydroxyacyl acid intermediaries in the fatty acid synthase cycle, to produce HAA. Four congeners of varying chain lengths were identified and quantified by UHPLC-QTOF mass spectrometry and gas chromatography, including C10-C10 and C10-C8, and the less abundant C10-C12 and C10-C6 congeners. HAA was present in the intracellular fraction, but also showed increased accumulation in the extracellular medium. Moreover, HAA production was also observed under photoautotrophic conditions based on atmospheric CO₂. These results establish that RhIA is active in the chloroplast and is able to produce a new pool of HAA in a eukaryotic host. Subsequent engineering of microalgal strains should contribute to the development of an alternative clean, safe and cost-effective platform for the sustainable production of RLs.

Circadian rhythm promotes the biomass and amylose hyperaccumulation by mixotrophic cultivation of marine microalga *Platymonas helgolandica*

Autor: Qianwen Shi

Biotechnol Biofuels Bioprod. 2022 Jul 6;15(1):75. doi: 10.1186/s13068-022-02174-2.

ABSTRACT

BACKGROUND: Microalgal starch can be exploited for bioenergy, food, and bioplastics. Production of starch by green algae has been concerned for many years. Currently commonly used methods such as nutrient stress will affect cell growth, thereby inhibiting the production efficiency and quality of starch production. Simpler and more efficient control strategies need to be developed.

RESULT: We proposed a novel regulation method to promote the growth and starch accumulation by a newly isolated Chlorophyta *Platymonas helgolandica*. By adding exogenous glucose and controlling the appropriate circadian light and dark time, the highest dry weight accumulation 6.53 g L⁻¹ (Light:Dark = 12:12) can be achieved, and the highest starch concentration could reach 3.88 g L⁻¹ (Light:Dark = 6:18). The highest production rate was 0.40 g L⁻¹ d⁻¹ after 9 days of production. And this method helps to improve the ability to produce amylose, with the highest accumulation of 39.79% DW amylose. We also discussed the possible mechanism of this phenomenon through revealing changes in the mRNA levels of key genes.



CONCLUSION: This study provides a new idea to regulate the production of amylose by green algae. For the first time, it is proposed to combine organic carbon source addition and circadian rhythm regulation to increase the starch production from marine green alga. A new starch-producing microalga has been isolated that can efficiently utilize organic matter and grow with or without photosynthesis.

Co-generation of biohydrogen and biochemicals from co-digestion of *Chlorella* sp. biomass hydrolysate with sugarcane leaf hydrolysate in an integrated circular biorefinery concept

Autor: Napapat Sitthikitpanya

Biotechnol Biofuels. 2021 Oct 1;14(1):197. doi: 10.1186/s13068-021-02041-6.

ABSTRACT

BACKGROUND: A platform for the utilization of the *Chlorella* sp. biomass and sugarcane leaves to produce multiple products (biorefinery concept) including hydrogen, methane, polyhydroxyalkanoates (PHAs), lipid, and soil supplement with the goal to achieve the zero waste generation (circular economy) is demonstrated in this study. Microalgal biomass were hydrolyzed by mixed enzymes while sugarcane leaves were pretreated with alkali followed by enzyme. Hydrolysates were used to produce hydrogen and the hydrogenic effluent was used to produce multi-products. Solid residues at the end of hydrogen fermentation and the remaining acidified slurries from methane production were evaluated for the compost properties.

RESULTS: The maximum hydrogen yield of 207.65 mL-H₂/g-volatile solid (VS) added was obtained from 0.92, 15.27, and 3.82 g-VS/L of *Chlorella* sp. biomass hydrolysate, sugarcane leaf hydrolysate, and anaerobic sludge, respectively. Hydrogenic effluent produced 321.1 mL/g-VS of methane yield, 2.01 g/L PHAs concentration, and 0.20 g/L of lipid concentration. Solid residues and the acidified slurries at the end of the hydrogen and methane production process were proved to have compost properties.

CONCLUSION: Hydrogen production followed by methane, PHA and lipid productions is a successful integrated circular biorefinery platform to efficiently utilize the hydrolysates of *Chlorella* sp. biomass and sugarcane leaf. The potential use of the solid residues at the end of hydrogen fermentation and the remaining acidified slurries from methane production as soil supplements demonstrates the zero waste concept. The approach revealed in this study provides a foundation for the optimal use of feedstock, resulting in zero waste.

Comparative Assessment of Nitrogen Concentration Effect on Microalgal Growth and Biochemical Characteristics of Two *Chlorella* Strains Cultivated in Digestate

Autor: Savvas Giannis Mastropetros

Mar Drugs. 2022 Jun 25;20(7):415. doi: 10.3390/md20070415.

ABSTRACT

Microalgae have been recently recognized as a promising alternative for the effective treatment of anaerobic digestion effluents. However, to date, a widely applied microalgae-based process is still absent, due to several constraints mainly attributed to high ammonia concentrations and turbidity, both hindering microalgal growth. Within this scope, the purpose of the present study was to investigate the performance of two *Chlorella* strains, SAG 211-11b and a local Algerian isolate, under different nitrogen levels, upon ammonia stripping. The experiments were performed on cylindrical photobioreactors under controlled pH (7.8 ± 0.2) and temperature (25 ± 2 °C). Cultures were monitored for biomass production and substrate consumption. After sampling at the beginning of the stationary phase of growth (12th day) and after the maturation of the cells (24th day), an analysis of the produced biomass was conducted, in terms of its biochemical components. The local isolate grew better than *C. vulgaris* 211-11b, resulting in 1.43 mg L⁻¹ biomass compared to 1.02 mg L⁻¹ under 25 mg NH₄-N L⁻¹, while organic carbon and nutrient consumption varied between the two strains and different conditions. Concerning biomass quality, a high initial NH₄-N concentration led to high protein content, while low nitrogen levels favored fatty acid (FA) accumulation, though the production of pigments was inhibited. In particular, the protein content of the final biomass was determined close to 45% of the dry weight in all experimental scenarios with adequate nitrogen, while proteins



decreased, and the fatty acids approached 20% in the case of the local isolate grown on the substrate with the lowest initial ammonium nitrogen (25 mg NH₄-N L⁻¹). The novelty of the present work lies in the comparison of a microalga with industrial applications against a local isolate of the same species, which may prove to be even more robust and profitable.

Coupling of Microalgae Cultivation with Anaerobic Digestion of Poultry Wastes: Toward Sustainable Value Added Bioproducts

Autor: Rajinikanth Rajagopal

Bioengineering (Basel). 2021 May 4;8(5):57. doi: 10.3390/bioengineering8050057.

ABSTRACT

Third generation biofuels and high-value bioproducts produced from microalgal biomass have been considered promising long-term sustainable alternatives for energy and/or food production, potentially decreasing greenhouse gas emissions. Microalgae as a source of biofuels have been widely studied for bioethanol/biodiesel/biogas production. However, critical research is needed in order to increase the efficiency of microalgae production from high-N agri-waste, not only for biofuels but also for bio-based products, and thus enhance its commercial viability. The growth in the poultry industry has led to increased chicken manure (CM), which are rich in ammonia, phosphate, potassium, and other trace elements. These constituents could be used as nutrients for growing microalgae. In this research, a two-stage (liquid-solid) anaerobic digester treating CM at 20 ± 1 °C was performed, and liquid digestate (leachate) obtained after the digestion process was used as a substrate to grow the microalgal strain *Chlorella vulgaris* CPCC 90. Considering the high-N content (NH₃-N: 5314 mg/L; TKN: 6197 mg/L) in liquid digestate, different dilutions were made, using distilled water to obtain viz. 10%, 30%, 50%, 70%, 90%, and 100% of the digestate concentrations for the microalgae cultivation. Preliminary results showed that *Chlorella vulgaris* CPCC 90 was able to grow and utilize nutrients from a 10% diluted CM digestate. Future research is underway to enhance microalgal growth at higher digestate concentrations and to optimize the use of microalgae/microalgae-bacteria consortia for better adaptation to high-N content wastes. An AD-microalgae coupling scenario has been proposed for the circulation bioeconomy framework.

Effect of untreated and pretreated sugarcane molasses on growth performance of *Haematococcus pluvialis* microalgae in inorganic fertilizer and macrophyte extract culture media

Autor: L H Sipaúba-Tavares

Braz J Biol. 2022 Aug 29;82:e263282. doi: 10.1590/1519-6984.263282. eCollection 2022.

ABSTRACT

The growth of *Haematococcus pluvialis* in two alternative culture media NPK (10:10:10) and ME (macrophyte extract), under mixotrophic conditions using sugarcane molasses as a carbon source were evaluated for 28 days. The molasses was used in two different ways, in a native form (untreated) and a hydrolyzed (pretreated). Cell density of *Haematococcus pluvialis* in mixotrophic cultivation was higher in pretreated molasses. Growth rate was higher when pretreated molasses were employed in mixotrophic cultivation with NPK culture medium ($k=0.5$ 7th growth day). Biomass, chlorophyll-a, conductivity and total inorganic nitrogen were not significantly different ($p>0.05$) during the experimental period for two mixotrophic cultivation and culture media. Protein contents of *H. pluvialis* biomass were higher in NPK culture medium with pretreated molasses (50% dry biomass). Annual biomass production was 520 kg⁻¹ dry biomass with untreated molasses for two culture media, and 650 and 520 kg⁻¹ dry biomass with pretreated molasses for NPK and ME culture media, respectively. The use of NPK and ME culture media in mixotrophic cultivation may be a new protocol for *H. pluvialis* cultivation due to the low cost and similar annual production.



Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of microorganisms obtained through synthetic biology

Autor: EFSA Scientific Committee

EFSA J. 2022 Aug 16;20(8):e07479. doi: 10.2903/j.efsa.2022.7479. eCollection 2022 Aug.

ABSTRACT

EFSA was asked by the European Commission to evaluate synthetic biology (SynBio) developments for agri-food use in the near future and to determine whether or not they are expected to constitute potential new hazards/risks. Moreover, EFSA was requested to evaluate the adequacy of existing guidelines for risk assessment of SynBio and if updated guidance is needed. The scope of this Opinion covers food and feed risk assessment, the variety of microorganisms that can be used in the food/feed chain and the whole spectrum of techniques used in SynBio. This Opinion complements a previously adopted Opinion with the evaluation of existing guidelines for the microbial characterisation and environmental risk assessment of microorganisms obtained through SynBio. The present Opinion confirms that microbial SynBio applications for food and feed use, with the exception of xenobionts, could be ready in the European Union in the next decade. New hazards were identified related to the use or production of unusual and/or new-to-nature components. Fifteen cases were selected for evaluating the adequacy of existing guidelines. These were generally adequate for assessing the product, the production process, nutritional and toxicological safety, allergenicity, exposure and post-market monitoring. The comparative approach and a safety assessment per se could be applied depending on the degree of familiarity of the SynBio organism/product with the non-genetically modified counterparts. Updated guidance is recommended for: (i) bacteriophages, protists/microalgae, (ii) exposure to plant protection products and biostimulants, (iii) xenobionts and (iv) feed additives for insects as target species. Development of risk assessment tools is recommended for assessing nutritional value of biomasses, influence of microorganisms on the gut microbiome and the gut function, allergenic potential of new-to-nature proteins, impact of horizontal gene transfer and potential risks of living cell intake. A further development towards a strain-driven risk assessment approach is recommended.

Exploring Exogenous Indole-3-acetic Acid's Effect on the Growth and Biochemical Profiles of *Synechocystis* sp. PAK13 and *Chlorella variabilis*

Autor: Wael A Fathy

Molecules. 2023 Jul 19;28(14):5501. doi: 10.3390/molecules28145501.

ABSTRACT

Microalgae have garnered scientific interest for their potential to produce bioactive compounds. However, the large-scale industrial utilization of microalgae faces challenges related to production costs and achieving optimal growth conditions. Thus, this study aimed to investigate the potential role of exogenous indole-3-acetic acid (IAA) application in improving the growth and production of bioactive metabolites in microalgae. To this end, the study employed different concentrations of exogenously administered IAA ranging from 0.36 μM to 5.69 μM to assess its influence on the growth and biochemical composition of *Synechocystis* and *Chlorella*. IAA exposure significantly increased IAA levels in both strains. Consequentially, improved biomass accumulation in parallel with increased total pigment content by approximately eleven-fold in both strains was observed. Furthermore, the application of IAA stimulated the accumulation of primary metabolites. Sugar levels were augmented, providing a carbon source that facilitated amino acid and fatty acid biosynthesis. As a result, amino acid levels were enhanced as well, leading to a 1.55-fold increase in total amino acid content in *Synechocystis* and a 1.42-fold increase in *Chlorella*. Total fatty acids content increased by 1.92-fold in *Synechocystis* and by 2.16-fold in *Chlorella*. Overall, the study demonstrated the effectiveness of exogenously adding IAA as a strategy for enhancing the accumulation of microalgae biomass and biomolecules. These findings contribute to the advancement of microalgae-based technologies, opening new avenues to produce economically important compounds derived from microalgae.

Extracts from *Chlorella vulgaris* Protect Mesenchymal Stromal Cells from Oxidative Stress Induced by Hydrogen Peroxide



ABSTRACT

Microalgae as unicellular eukaryotic organisms demonstrate several advantages for biotechnological and biological applications. Natural derived microalgae products demand has increased in food, cosmetic and nutraceutical applications lately. The natural antioxidants have been used for attenuation of mitochondrial cell damage caused by oxidative stress. This study evaluates the in vitro protective effect of *Chlorella vulgaris* bioactive extracts against oxidative stress in human mesenchymal stromal/stem cells (MSCs). The classical solid-liquid and the supercritical extraction, using biomass of commercially available and laboratory cultivated *C. vulgaris*, are employed. Oxidative stress induced by 300 μ M H₂O₂ reduces cell viability of MSCs. The addition of *C. vulgaris* extracts, with increased protein content compared to carbohydrates, to H₂O₂ treated MSCs counteracted the oxidative stress, reducing reactive oxygen species levels without affecting MSC proliferation. The supercritical extraction was the most efficient extraction method for carotenoids resulting in enhanced antioxidant activity. Pre-treatment of MSCs with *C. vulgaris* extracts mitigates the oxidative damage ensued by H₂O₂. Initial proteomic analysis of secretome from licensed (TNF α -activated) MSCs treated with algal extracts reveals a signature of differentially regulated proteins that fall into clinically relevant pathways such as inflammatory signaling. The enhanced antioxidative and possibly anti-inflammatory capacity could be explored in the context of future cell therapies.

Filter cake extract from the beet sugar industry as an economic growth medium for the production of *Spirulina platensis* as a microbial cell factory for protein

Autor: Sara Saad
Microb Cell Fact. 2023 Jul 24;22(1):136. doi: 10.1186/s12934-023-02146-7.

ABSTRACT

BACKGROUND: Beet filter cake (BFC) is a by-product of sugar beet processing, which is difficult to dispose of and involves severe environmental concerns. *Spirulina platensis* is a microalga with a high protein content essential for human and animal nutrition. The present study aimed to utilize the beet filter cake extract (BFCE) to produce *Spirulina platensis* commercially. However, the cultivation of *S. platensis* on BFCE to produce economically single-cell protein has not been reported previously.

RESULTS: The batch experiment revealed the maximum dry weight at Zarrouk's medium (0.4 g/L) followed by 0.34 g/L in the treatment of 75% BFCE. The highest protein content was 50% in Zarrouk's medium, followed by 46.5% in 25% BFCE. However, adding a higher concentration of 100% BFCE led to a protein content of 31.1%. In the adaption experiment, *S. platensis* showed an increase in dry cell weight and protein content from 25 to 75% BFCE (0.69 g/L to 1.12 g/L and 47.0% to 52.54%, respectively) with an insignificant variation compared to Zarrouk's medium ($p \leq 0.05$), indicating that *S. platensis* can be economically produced when cultivated on 75% BFCE. The predicated parameters from response surface methodology were NaNO₃ (2.5 g/L), NaHCO₃ (0.67 g/L), BFCE (33%) and pH = 8, which resulted in biomass yield and protein content (0.56 g/L and 52.5%, respectively) closer to that achieved using the standard Zarrouk's medium (0.6 g/L and 55.11%). Moreover, the total essential amino acid content was slightly higher in the optimized medium (38.73%) than SZM (36.98%).

CONCLUSIONS: Therefore, BFCE supplemented medium could be used as a novel low-cost alternative growth medium for producing a single-cell protein with acceptable quantity and quality compared to the standard Zarrouk's medium.

Folate-mediated one-carbon metabolism as a potential antifungal target for the sustainable cultivation of microalga *Haematococcus pluvialis*

Autor: Hailong Yan
Biotechnol Biofuels Bioprod. 2023 Jun 17;16(1):104. doi: 10.1186/s13068-023-02353-9.

ABSTRACT

BACKGROUND: Microalgae are widely considered as multifunctional cell factories that are able to transform the photo-synthetically fixed CO₂ to numerous high-value compounds, including lipids, carbohydrates, proteins and pigments. However, contamination of the algal mass culture with fungal parasites continues to threaten the production of algal biomass, which dramatically highlights the importance of developing effective measures to control the fungal infection. One viable solution is to identify potential metabolic pathways that are essential for fungal pathogenicity but are not obligate for algal growth, and to use inhibitors targeting such pathways to restrain the infection. However, such targets remain largely unknown, making it challenging to develop effective measures to mitigate the infection in algal mass culture.

RESULTS: In the present study, we conducted RNA-Seq analysis for the fungus *Paraphysoderma sedebokerense*, which can infect the astaxanthin-producing microalga *Haematococcus pluvialis*. It was found that many differentially expressed genes (DEGs) related to folate-mediated one-carbon metabolism (FOCM) were enriched in *P. sedebokerense*, which was assumed to produce metabolites required for the fungal parasitism. To verify this hypothesis, antifolate that hampered FOCM was applied to the culture systems. Results showed that when 20 ppm of the antifolate co-trimoxazole were added, the infection ratio decreased to ~ 10% after 9 days inoculation (for the control, the infection ratio was 100% after 5 days inoculation). Moreover, application of co-trimoxazole to *H. pluvialis* mono-culture showed no obvious differences in the biomass and pigment accumulation compared with the control, suggesting that this is a potentially algae-safe, fungi-targeted treatment.

CONCLUSIONS: This study demonstrated that applying antifolate to *H. pluvialis* culturing systems can abolish the infection of the fungus *P. sedebokerense* and the treatment shows no obvious disturbance to the algal culture, suggesting FOCM is a potential target for antifungal drug design in the microalgal mass culture industry.

Galdieria sulphuraria: An Extremophilic Alga as a Source of Antiviral Bioactive Compounds

Autor: Annalisa Ambrosino

Mar Drugs. 2023 Jun 28;21(7):383. doi: 10.3390/md21070383.

ABSTRACT

In the last decades, the interest in bioactive compounds derived from natural sources including bacteria, fungi, plants, and algae has significantly increased. It is well-known that aquatic or terrestrial organisms can produce, in special conditions, secondary metabolites with a wide range of biological properties, such as anticancer, antioxidant, anti-inflammatory, and antimicrobial activities. In this study, we focused on the extremophilic microalga *Galdieria sulphuraria* as a possible producer of bioactive compounds with antiviral activity. The algal culture was subjected to organic extraction with acetone. The cytotoxicity effect of the extract was evaluated by the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The antiviral activity was assessed through a plaque assay against herpesviruses and coronaviruses as enveloped viruses and poliovirus as a naked one. The monolayer was treated with different concentrations of extract, ranging from 1 µg/mL to 200 µg/mL, and infected with viruses. The algal extract displayed strong antiviral activity at non-toxic concentrations against all tested enveloped viruses, in particular in the virus pre-treatment against HSV-2 and HCoV-229E, with IC₅₀ values of 1.7 µg/mL and IC₉₀ of 1.8 µg/mL, respectively. However, no activity against the non-enveloped poliovirus has been detected. The inhibitory effect of the algal extract was confirmed by the quantitative RT-PCR of viral genes. Preliminary chemical profiling of the extract was performed using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS), revealing the enrichment in primary fatty acid amides (PFAA), such as oleamide, palmitamide, and pheophorbide A. These promising results pave the way for the further purification of the mixture to explore its potential role as an antiviral agent.

Genetic engineering for biohydrogen production from microalgae

Autor: Jiaqi Zhang

iScience. 2023 Jul 3;26(8):107255. doi: 10.1016/j.isci.2023.107255. eCollection 2023 Aug 18.

ABSTRACT



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The development of biohydrogen as an alternative energy source has had great economic and environmental benefits. Hydrogen production from microalgae is considered a clean and sustainable energy production method that can both alleviate fuel shortages and recycle waste. Although algal hydrogen production has low energy consumption and requires only simple pretreatment, it has not been commercialized because of low product yields. To increase microalgal biohydrogen production several technologies have been developed, although they struggle with the oxygen sensitivity of the hydrogenases responsible for hydrogen production and the complexity of the metabolic network. In this review, several genetic and metabolic engineering studies on enhancing microalgal biohydrogen production are discussed, and the economic feasibility and future direction of microalgal biohydrogen commercialization are also proposed.

Genome editing with removable TALEN vectors harboring a yeast centromere and autonomous replication sequence in oleaginous microalga

Autor: Tomokazu Kurita

Sci Rep. 2022 Feb 15;12(1):2480. doi: 10.1038/s41598-022-06495-y.

ABSTRACT

Algal lipids are expected to become a basis for sustainable fuels because of the highly efficient lipid production by photosynthesis accompanied by carbon dioxide assimilation. Molecular breeding of microalgae has been studied to improve algal lipid production, but the resultant gene-modified algae containing transgenes are rarely used for outdoor culture because the use of genetically modified organisms (GMOs) is strictly restricted under biocontainment regulations. Recently, it was reported that plasmids containing yeast centromere and autonomous replication sequence (CEN/ARS) behaved as episomes in *Nannochloropsis* species. We previously reported that the Platinum TALEN (PtTALEN) system exhibited high activity in *Nannochloropsis oceanica*. Therefore, we attempted to develop a genome editing system in which the expression vectors for PtTALEN can be removed from host cells after introduction of mutations. Using all-in-one PtTALEN plasmids containing CEN/ARS, targeted mutations and removal of all-in-one vectors were observed in *N. oceanica*, suggesting that our all-in-one PtTALEN vectors enable the construction of mutated *N. oceanica* without any transgenes. This system will be a feasible method for constructing non-GMO high-performance algae.

Genome-centric investigation of anaerobic digestion using sustainable second and third generation substrates

Autor: Roland Wirth

J Biotechnol. 2021 Sep 20;339:53-64. doi: 10.1016/j.jbiotec.2021.08.002. Epub 2021 Aug 8.

ABSTRACT

Biogas production through co-digestion of second and third generation substrates is an environmentally sustainable approach. Green willow biomass, chicken manure waste and microalgae biomass substrates were combined in the anaerobic digestion experiments. Biochemical methane potential test showed that biogas yields of co-digestions were significantly higher compared to the yield when energy willow was the sole substrate. To scale up the experiment continuous stirred-tank reactors (CSTRs) are employed, digestion parameters are monitored. Furthermore, genome-centric metagenomics approach was employed to gain functional insight into the complex anaerobic decomposing process. This revealed the importance of Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes phyla as major bacterial participants, while Methanomicrobia and Methanobacteria represented the archaeal constituents of the communities. The bacterial phyla were shown to perform the carbohydrate hydrolysis. Among the representatives of long-chain carbohydrate hydrolysing microbes Bin_61: Clostridia is newly identified metagenome assembled genome (MAG) and Bin_13: DTU010 sp900018335 is common and abundant in all CSTRs. Methanogenesis was linked to the slow-growing members of the community, where hydrogenotrophic methanogen species *Methanoculleus* (Bin_10) and *Methanobacterium* (Bin_4) predominate. A sensitive balance between H₂ producers and consumers was shown to be critical for stable biomethane production and efficient waste biodegradation.



Heterotrophic growth of *Galdieria sulphuraria* on residues from aquaculture and fish processing industries

Autor: Daniel Pleissner

Bioresour Technol. 2023 Sep;384:129281. doi: 10.1016/j.biortech.2023.129281. Epub 2023 Jun 8.

ABSTRACT

The study aimed at zero-waste utilization of fish processing streams for cultivation of microalgae *Galdieria sulphuraria*. Wastewater from a fish processing facility, slam (mix of used fish feed and faeces), and dried pellet (sediments after enzymatic hydrolysis of rainbow trout) were investigated as potential sources of carbon, nitrogen, and phosphate for cultivation of *G. sulphuraria*. The pellet extract was found to support the growth of *G. sulphuraria* when appropriately diluted, at concentrations below 40 % (v/v). It was revealed that wastewater does not impact the growth negatively, however free amino nitrogen and carbon sources need to be supplied from another source. Therefore, only proteolyzed pellet extract (20 %, v/v) was selected for upscaling and a biomass concentration of 80 g L⁻¹ (growth rate was 0.72 day⁻¹) was achieved in a non-sterile fed-batch culture. Even though biomass was produced under non-sterile conditions no pathogens such as *Salmonella* sp. could be detected.

Hyperhalophilic Diatom Extract Protects against Lead-Induced Oxidative Stress in Rats and Human HepG2 and HEK293 Cells

Autor: Wassim Guermazi

Pharmaceuticals (Basel). 2023 Jun 13;16(6):875. doi: 10.3390/ph16060875.

ABSTRACT

This work investigated the protective effects of microalga *Halamphora* sp. extract (HExt), a nutraceutical and pharmacological natural product, on human lead-intoxicated liver and kidney cells in vitro and in vivo in Wistar rats. The human hepatocellular carcinoma cell line HepG2 and the human embryonic kidney cell line HEK293 were used for the in vitro study. The analysis of the fatty acid methyl esters in the extract was performed via GC/MS. The cells were pretreated with HExt at 100 µg mL⁻¹, followed by treatment with different concentrations of lead acetate, ranging from 25 to 200 µM for 24 h. The cultures were incubated (5% CO₂, 37 °C) for 24 h. Four groups, each containing six rats, were used for the in vivo experiment. The rats were exposed to subchronic treatment with a low dose of lead acetate (5 mg kg⁻¹ b.w. per day). Pretreating HepG2 and HEK293 cells with the extract (100 µg mL⁻¹) significantly ($p < 0.05$) protected against the cytotoxicity induced by lead exposure. For the in vivo experiment, the biochemical parameters in serum—namely, the level of malondialdehyde (MDA), and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—were measured in the organ homogenate supernatants. HExt was found to be rich in fatty acids, mainly palmitic and palmitoleic acids (29.464% and 42.066%, respectively). In both the in vitro and in vivo experiments, cotreatment with HExt protected the liver and kidney cell structures and significantly preserved the normal antioxidant and biochemical parameters in rats. This study discovered the possible protective effect of HExt, which could be beneficial for Pb-intoxicated cells.

Improving biomass and carbohydrate production of microalgae in the rotating cultivation system on natural carriers

Autor: Zahra Mousavian

AMB Express. 2023 Apr 29;13(1):39. doi: 10.1186/s13568-023-01548-5.

ABSTRACT

Biofilm-based algal technologies have gained popularity due to higher biomass productivity, efficient harvesting, and water-saving over suspended growth systems. A rotating attached system was designed to assess the biofilm-forming capacity of different



isolated microalgal strains from the Persian Gulf. Four microalgal strains, including two *Chlorella* sp., one *Picochlorum* sp. and one filamentous cyanobacterium *Desmonostoc* sp. were cultivated on four carriers: jute, cotton, yarn and nylon. The carriers' physicochemical surface characteristics and attachment effects, like contact angle, were investigated. The incorporated biomass and exopolysaccharides (EPS) content in the suspended and biofilm system was calculated and compared. The results showed that the cyanobacterium strain had the biofilm formation capability on both jute and cotton in the attached cultivation system. Under the same culture conditions, the biomass productivity on jute and cotton carriers was significantly higher (4.76 and 3.61 g m⁻² respectively) than the growth in aqueous suspension (1.19 g m⁻² d⁻¹). The greatest incorporated exopolysaccharides amount was observed on jute (43.62 ± 4.47%) and the lowest amount was obtained from the growth on positive charge yarn (18.62 ± 1.88%). This study showed that in comparison with planktonic growth, the colonization of cyanobacterial cells and subsequent production of extracellular matrix and biofilm formation can lead to increased biomass production.

Influence of culture media composition on the rheology of microalgae concentrates on a large scale

Autor: Solaima Belachqer-El Attar

N Biotechnol. 2023 Jul 31;77:90-99. doi: 10.1016/j.nbt.2023.07.005.

ABSTRACT

The role of microalgae in the production of bioproducts and biofuels, along with their ability to provide a sustainable pathway for wastewater treatment, makes them promising alternatives to conventional processes. Nevertheless, large-scale downstream processing requires an understanding of biomass rheology that needs to be addressed further. This study aimed to characterize microalgal concentrates rheologically in different culture media. The presence of bacteria was quantified by photorespirometry and plate counting techniques. The culture medium was found to significantly influence viscosity, with primary wastewater exhibiting the highest viscosity and seawater plus pig slurry the lowest. The concentration of heterotrophic bacteria was directly related to the viscosity. Extracellular polysaccharides (EPS) in supernatant exhibited an inverse viscosity trend compared to biomass concentrates, with pig slurry cultures having higher concentrations. These findings emphasize the profound influence of culture medium and EPS on the rheology of microalgal biomass, underscoring the need for continued research aimed at facilitating and optimizing large-scale downstream processes within the framework of a circular economy and the attainment of the Sustainable Development Goals (6,8, and 12).

Interactive Effects of Ceftriaxone and Chitosan Immobilization on the Production of Arachidonic Acid by and the Microbiome of the Chlorophyte *Lobosphaera* sp. IPPAS C-2047

Autor: Svetlana Vasilieva

Int J Mol Sci. 2023 Jul 1;24(13):10988. doi: 10.3390/ijms241310988.

ABSTRACT

Pharmaceuticals including antibiotics are among the hazardous micropollutants (HMP) of the environment. Incomplete degradation of the HMP leads to their persistence in water bodies causing a plethora of deleterious effects. Conventional wastewater treatment cannot remove HMP completely and a promising alternative comprises biotechnologies based on microalgae. The use of immobilized microalgae in environmental biotechnology is advantageous since immobilized cultures allow the recycling of the microalgal cells, support higher cell densities, and boost tolerance of microalgae to stresses including HMP. Here, we report on a comparative study of HMP (exemplified by the antibiotic ceftriaxone, CTA) removal by suspended and chitosan-immobilized cells of *Lobosphaera* sp. IPPAS C-2047 in flasks and in a column bioreactor. The removal of CTA added in the concentration of 20 mg/L was as high as 65% (in the flasks) or 85% (in the bioreactor). The adsorption on the carrier and abiotic oxidation were the main processes contributing 65-70% to the total CTA removal, while both suspended and immobilized cells took up 25-30% of CTA. Neither the immobilization nor CTA affected the accumulation of arachidonic acid (ARA) by *Lobosphaera* sp. during bioreactor tests but the subsequent nitrogen deprivation increased



ARA accumulation 2.5 and 1.7 times in the suspended and chitosan-immobilized microalgae, respectively. The study of the *Lobosphaera* sp. microbiome revealed that the immobilization of chitosan rather than the CTA exposure was the main factor displacing the taxonomic composition of the microbiome. The possibility and limitations of the use of chitosan-immobilized *Lobosphaera* sp. IPPAS C-2047 for HMP removal coupled with the production of valuable long-chain polyunsaturated fatty acids is discussed.

Lipid extraction and analysis of microalgae strain *Pectinodesmus* PHM3 for biodiesel production

Autor: Dania Akram Kiyani

BMC Biotechnol. 2023 Jul 10;23(1):20. doi: 10.1186/s12896-023-00784-8.

ABSTRACT

The current study is focused on the lipid extract of microalgae; *Pectinodesmus* strain PHM3 and its general analysis in terms of chemical contents. Combinations of both chemical and mechanistic approaches were applied to obtain the maximum yield of lipids which was recorded to be 23% per gram through continuous agitation using Folch solution. The extraction methods used in this study included: Bligh and Dyers method, Continuous agitation method, Extraction using Soxhlet and Acid base extraction method. Lipid quantification of ethanol and Folch solution lipid extract was performed through gravimetric methods and qualification was done through Fourier Transmission Infrared Spectroscopy (FTIR) and Gas Chromatography-mass spectrometry (GC-MS). Phytochemical analysis identified other compounds in ethanol extract and the results confirmed the presence of steroids, coumarins, tannins, phenols and carbohydrates. Transesterification of lipids showed 7% per gram dry weight yield of *Pectinodesmus* PHM3. GC-MS studies of extracted biodiesel suggested that 72% of biofuels was in the form of dipropyl ether, ethyl butyl ethers, methyl butyl ether and propyl butyl ether. Lipid processing of acid-base extract showed that oily nature of lipid shifted to a more precipitated form which is a common observation when mixture of lipids is converted to phosphatides.

Machine learning-informed and synthetic biology-enabled semi-continuous algal cultivation to unleash renewable fuel productivity

Autor: Bin Long

Nat Commun. 2022 Jan 27;13(1):541. doi: 10.1038/s41467-021-27665-y.

ABSTRACT

Algal biofuel is regarded as one of the ultimate solutions for renewable energy, but its commercialization is hindered by growth limitations caused by mutual shading and high harvest costs. We overcome these challenges by advancing machine learning to inform the design of a semi-continuous algal cultivation (SAC) to sustain optimal cell growth and minimize mutual shading. An aggregation-based sedimentation (ABS) strategy is then designed to achieve low-cost biomass harvesting and economical SAC. The ABS is achieved by engineering a fast-growing strain, *Synechococcus elongatus* UTEX 2973, to produce limonene, which increases cyanobacterial cell surface hydrophobicity and enables efficient cell aggregation and sedimentation. SAC unleashes cyanobacterial growth potential with 0.1 g/L/hour biomass productivity and 0.2 mg/L/hour limonene productivity over a sustained period in photobioreactors. Scaling-up the SAC with an outdoor pond system achieves a biomass yield of 43.3 g/m²/day, bringing the minimum biomass selling price down to approximately \$281 per ton.

Manipulation in Culture Conditions of *Nanofrustulum shiloi* for Enhanced Fucoxanthin Production and Isolation by Preparative Chromatography

Autor: Ayşegül Erdoğan

Molecules. 2023 Feb 20;28(4):1988. doi: 10.3390/molecules28041988.

ABSTRACT



Microalgae produce a variety of high-value chemicals including carotenoids. Fucoxanthin is also a carotenoid that has many physiological functions and biological properties. For this reason, the cost-effective production of fucoxanthin at an industrial scale has gained significant attention. In the proposed study, fucoxanthin production was aimed to be increased by altering the culture conditions of *N. shiloi*. The effect of light intensity, aeration rate, different nitrogen sources, and oxidative stress on the biomass and fucoxanthin productivity have been discussed. Based on these results, the fucoxanthin increased to 97.45 ± 2.64 mg/g by adjusting the light intensity to 50 $\mu\text{mol}/\text{m}^2\text{s}$, and aeration rate at 5 L/min using oxidative stress through the addition of 0.1 mM H_2O_2 and 0.1 mM NaOCl to the culture medium. Fucoxanthin was then purified with preparative HPLC using C30 carotenoid column (10 mm \times 250 mm, 5 μm). After the purification procedure, Liquid chromatography tandem mass spectrometry (LC-MS/MS) and UV-vis spectroscopy were employed for the confirmation of fucoxanthin. This study presented a protocol for obtaining and purifying considerable amounts of biomass and fucoxanthin from diatom by manipulating culture conditions. With the developed methodology, *N. shiloi* could be evaluated as a promising source of fucoxanthin at the industrial scale for food, feed, cosmetic, and pharmaceutical industries.

Mechanism and kinetic model of microalgal enzymatic hydrolysis for prospective bioethanol conversion

Autor: None Padil

RSC Adv. 2023 Jul 17;13(31):21403-21413. doi: 10.1039/d3ra01556d. eCollection 2023 Jul 12.

ABSTRACT

Tetraselmis chuii is a potential microalgae that is in consideration for producing bioethanol owing to its large content of carbohydrates. The glucose production from *T. chuii* through an enzymatic process with cellulase and xylanase (pretreatment process) and α -amylase and glucoamylase (saccharification process) was studied. The mechanism of the enzymatic process was developed and the kinetic models were then evaluated. For the pretreatment process, enzymes with 30% concentration reacted at 30 °C for 40 min resulted in 35.9% glucose yield. For the saccharification process, the highest glucose yield of 90.03% was obtained using simultaneous α -amylase (0.0006%) and glucoamylase (0.01%) enzymes at 55 °C and for 40 min. The kinetic models fitted well with the experimental data. The model also revealed that the saccharification process performed better than the pretreatment process with a higher kinetic constant and lower activation energy. The proposed kinetic model plays an important role in implementing processes at a larger scale.

Metabolomic analysis reveals astaxanthin biosynthesis in heterotrophic microalga *Chromochloris zofingiensis*

Autor: Qiaohong Chen

Bioresour Technol. 2023 Apr;374:128811. doi: 10.1016/j.biortech.2023.128811. Epub 2023 Feb 28.

ABSTRACT

The utilization of gibberellic acid-3, high carbon/nitrogen ratio and salinity concentration can effectively enhance astaxanthin biosynthesis in *Chromochloris zofingiensis* under the heterotrophic conditions, but the underlying mechanisms remained yet to be investigated. The metabolomics analysis revealed that enhancement of the glycolysis, pentose phosphate pathways (PPP), and tricarboxylic acid (TCA) cycle led to astaxanthin accumulation under the induction conditions. The increased fatty acids can significantly increase astaxanthin esterification. The addition of appropriate concentrations of glycine (Gly) and γ -aminobutyric acid (GABA) promoted astaxanthin biosynthesis in *C. zofingiensis*, as well as benefiting for biomass yield. With the addition of 0.5 mM GABA, the astaxanthin yield increased to 0.35 g \cdot L $^{-1}$, which was 1.97-fold higher than that of the control. This study advanced understanding about astaxanthin biosynthesis in heterotrophic microalga, and provided novel strategies for enhanced astaxanthin production in *C. zofingiensis*.



Microalga *Coelastrella* sp. Cultivation on Unhydrolyzed Molasses-Based Medium towards the Optimization of Conditions for Growth and Biomass Production under Mixotrophic Cultivation

Autor: Kamolwan Thepsuthammarat

Molecules. 2023 Apr 20;28(8):3603. doi: 10.3390/molecules28083603.

ABSTRACT

Improving biomass production with the utilization of low-cost substrate is a crucial approach to overcome the hindrance of high cost in developing large-scale microalgae production. The microalga *Coelastrella* sp. KKU-P1 was mixotrophically cultivated using unhydrolyzed molasses as a carbon source, with the key environmental conditions being varied in order to maximize biomass production. The batch cultivation in flasks achieved the highest biomass production of 3.81 g/L, under an initial pH 5.0, a substrate to inoculum ratio of 100:3, an initial total sugar concentration of 10 g/L, and a sodium nitrate concentration of 1.5 g/L with continuous light illumination at 23.7 W/m². The photobioreactor cultivation results indicated that CO₂ supplementation did not improve biomass production. An ambient concentration of CO₂ was sufficient to promote the mixotrophic growth of the microalga as indicated by the highest biomass production of 4.28 g/L with 33.91% protein, 46.71% carbohydrate, and 15.10% lipid. The results of the biochemical composition analysis suggest that the microalgal biomass obtained is promising as a source of essential amino acids and pigments as well as saturated and monounsaturated fatty acids. This research highlights the potential for bioresource production via microalgal mixotrophic cultivation using untreated molasses as a low-cost raw material.

Microalgae Biomass as a New Potential Source of Sustainable Green Lubricants

Autor: Leonardo I Farfan-Cabrera

Molecules. 2022 Feb 11;27(4):1205. doi: 10.3390/molecules27041205.

ABSTRACT

Lubricants are materials able to reduce friction and/or wear of any type of moving surfaces facilitating smooth operations, maintaining reliable machine functions, and reducing risks of failures while contributing to energy savings. At present, most worldwide used lubricants are derived from crude oil. However, production, usage and disposal of these lubricants have significant impact on environment and health. Hence, there is a growing pressure to reduce demand of this sort of lubricants, which has fostered development and use of green lubricants, as vegetable oil-based lubricants (biolubricants). Despite the ecological benefits of producing/using biolubricants, availability of the required raw materials and agricultural land to create a reliable chain supply is still far from being established. Recently, biomass from some microalgae species has attracted attention due to their capacity to produce high-value lipids/oils for potential lubricants production. Thus, this multidisciplinary work reviews the main chemical-physical characteristics of lubricants and the main attempts and progress on microalgae biomass production for developing oils with pertinent lubricating properties. In addition, potential microalgae strains and chemical modifications to their oils to produce lubricants for different industrial applications are identified. Finally, a guide for microalgae oil selection based on its chemical composition for specific lubricant applications is provided.

Microalgae systems - environmental agents for wastewater treatment and further potential biomass valorisation

Autor: Helena M Amaro

J Environ Manage. 2023 Jul 1;337:117678. doi: 10.1016/j.jenvman.2023.117678. Epub 2023 Mar 21.

ABSTRACT



Water is the most valuable resource on the planet. However, massive anthropogenic activities generate threatening levels of biological, organic, and inorganic pollutants that are not efficiently removed in conventional wastewater treatment systems. High levels of conventional pollutants (carbon, nitrogen, and phosphorus), emerging chemical contaminants such as antibiotics, and pathogens (namely antibiotic-resistant ones and related genes) jeopardize ecosystems and human health. Conventional wastewater treatment systems entail several environmental issues: (i) high energy consumption; (ii) high CO₂ emissions; and (iii) the use of chemicals or the generation of harmful by-products. Hence, the use of microalgal systems (entailing one or several microalgae species, and in consortium with bacteria) as environmental agents towards wastewater treatment has been seen as an environmentally friendly solution to remove conventional pollutants, antibiotics, coliforms and antibiotic resistance genes. In recent years, several authors have evaluated the use of microalgal systems for the treatment of different types of wastewater, such as agricultural, municipal, and industrial. Generally, microalgal systems can provide high removal efficiencies of: (i) conventional pollutants, up to 99%, 99%, and 90% of total nitrogen, total phosphorus, and/or organic carbon, respectively, through uptake mechanisms, and (ii) antibiotics frequently found in wastewaters, such as sulfamethoxazole, ciprofloxacin, trimethoprim and azithromycin at 86%, 65%, 42% and 93%, respectively, through the most desirable microalgal mechanism, biodegradation. Although pathogens removal by microalgal species is complex and very strain-specific, it is also possible to attain total coliform and *Escherichia coli* removal of 99.4% and 98.6%, respectively. However, microalgal systems' effectiveness strongly relies on biotic and abiotic conditions, thus the selection of operational conditions is critical. While the combination of selected species (microalgae and bacteria), ratios and inoculum concentration allow the efficient removal of conventional pollutants and generation of high amounts of biomass (that can be further converted into valuable products such as biofuels and biofertilisers), abiotic factors such as pH, hydraulic retention time, light intensity and CO₂/O₂ supply also have a crucial role in conventional pollutants and antibiotics removal, and wastewater disinfection. However, some rationale must be considered according to the purpose. While alkaline pH induces the hydrolysis of some antibiotics and the removal of faecal coliforms, it also decreases phosphates solubility and induces the formation of ammonium from ammonia. Also, while CO₂ supply increases the removal of *E. coli* and *Pseudomonas aeruginosa*, as well as the microalgal growth (and thus the conventional pollutants uptake), it decreases *Enterococcus faecalis* removal. Therefore, this review aims to provide a critical review of recent studies towards the application of microalgal systems for the efficient removal of conventional pollutants, antibiotics, and pathogens; discussing the feasibility, highlighting the advantages and challenges of the implementation of such process, and presenting current case-studies of different applications of microalgal systems.

Microalgae to Bioenergy: Optimization of *Aurantiochytrium* sp. Saccharification

Autor: Joana Oliveira

Biology (Basel). 2023 Jun 29;12(7):935. doi: 10.3390/biology12070935.

ABSTRACT

Microalgae are a promising feedstock for bioethanol production, essentially due to their high growth rates and absence of lignin. Hydrolysis-where the monosaccharides are released for further fermentation-is considered a critical step, and its optimization is advised for each raw material. The present study focuses on the thermal acid hydrolysis (with sulfuric acid) of *Aurantiochytrium* sp. through a response surface methodology (RSM), studying the effect of acid concentration, hydrolysis time and biomass/acid ratio on both sugar concentration of the hydrolysate and biomass conversion yield. Preliminary studies allowed to establish the range of the variables to be optimized. The obtained models predicted a maximum sugar concentration (18.05 g/L; R² = 0.990) after 90 min of hydrolysis, using 15% (w/v) biomass/acid ratio and sulfuric acid at 3.5% (v/v), whereas the maximum conversion yield (12.86 g/100 g; R² = 0.876) was obtained using 9.3% (w/v) biomass/acid ratio, maintaining the other parameters. Model outputs indicate that the biomass/acid ratio and time are the most influential parameters on the sugar concentration and yield models, respectively. The study allowed to obtain a predictive model that is very well adjusted to the experimental data to find the best saccharification conditions for the *Aurantiochytrium* sp. microalgae.



Microalgal cultivation characteristics using commercially available air-cushion packaging material as a photobioreactor

Autor: Clifford R Merz

Sci Rep. 2023 Mar 7;13(1):3792. doi: 10.1038/s41598-023-30080-6.

ABSTRACT

Air-cushion (AC) packaging has become widely used worldwide. ACs are air-filled, dual plastic packaging solutions commonly found surrounding and protecting items of value within shipping enclosures during transit. Herein, we report on a laboratory assessment employing ACs as a microalgal photobioreactor (PBR). Such a PBR inherently addresses many of the operational issues typically encountered with open raceway ponds and closed photobioreactors, such as evaporative water loss, external contamination, and predation. Using half-filled ACs, the performance of microalgal species *Chlorella vulgaris*, *Nannochloropsis oculata*, and *Cyclotella cryptica* (diatom) was examined and the ash-free dry cell weight and overall biomass productivity determined to be 2.39 g/L and 298.55 mg/L/day for *N. oculata*, 0.85 g/L and 141.36 mg/L/day for *C. vulgaris*, and 0.67 g/L and 96.08 mg/L/day for *C. cryptica*. Furthermore, maximum lipid productivity of 25.54 mg/L/day AFDCW and carbohydrate productivity of 53.69 mg/L/day AFDCW were achieved by *C. cryptica*, while maximum protein productivity of 247.42 mg/L/day AFDCW was attained by *N. oculata*. Data from this work will be useful in determining the applicability and life-cycle profile of repurposed and reused ACs as potential microalgal photobioreactors depending upon the end product of interest, scale utilized, and production costs.

Mitigation of endogenous oxidative stress and improving growth, hemato-biochemical parameters, and reproductive performance of Zaraibi goat bucks by dietary supplementation with *Chlorella vulgaris* or/and vitamin C

Autor: A E Abdel-Khalek

Trop Anim Health Prod. 2023 Jul 13;55(4):267. doi: 10.1007/s11250-023-03657-6.

ABSTRACT

This study was conducted to explore the effects of dietary inclusion of *Chlorella vulgaris* (CV) or/and vitamin C (VC) on growth, hemato-biochemical parameters, oxidative and antioxidant status, reproductive hormones, and semen quality variables, and scrotal-testicular dimensions of Zaraibi goat bucks. Twenty sexually mature bucks (41.49 ± 0.91 kg BW) were randomly divided into 4 groups (5 bucks/group). The control group was fed the control diet, while the other three groups received a diet supplemented with VC (2 g/animal /day), CV (5 g/animal/day), and CV plus VC (the same levels), respectively, for 8 weeks (treatment period), and then semen was collected for 8 weeks. Results showed that dietary supplementation with CV-VC combination significantly increased the final body weight, weight gain, packed cell volume, hemoglobin, red blood cells, white blood cells, and lymphocytes; elevated serum total protein, globulin, testosterone, estradiol, superoxide dismutase, glutathione peroxidase with a significant reduction in Malondialdehyde in serum and seminal plasma. Also, the CV-VC combination significantly improved the ejaculate volume, total sperm output, sperm concentration, and live sperm, and reduced reaction time and sperm abnormality of bucks. Either CV or VC given separately or in combination, at the chosen levels, had no detrimental effects on animal physiological responses with normal hepatic and renal functions. Therefore, the CV-VC combination could be safely utilized as a dietary supplement in buck's diets to improve antioxidant defenses, scavenge free radicals, and potentiate buck's reproductive activities under normal conditions.

Nutritional Improvement of Fresh Cheese with Microalga *Chlorella vulgaris*: Impact on Composition, Structure and Sensory Acceptance

Autor: Rita Lousada Falcão

Food Technol Biotechnol. 2023 Jun;61(2):259-270. doi: 10.17113/ftb.61.02.23.7851.

ABSTRACT



Ministerio de Agricultura,
Ganadería y Pesca
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RESEARCH BACKGROUND: The production of foods fortified with bioactive ingredients has been recognized by food companies as a way to position their products in health food markets. The fortification of cheese represents a major challenge, due to the chemical and structural complexity of the cheese matrix, as well as the complexity of the biochemical reactions occurring during the fermentation and maturation processes. Microalgae are nutritious and sustainable food sources with important bioactive compounds such as proteins, polyunsaturated fatty acids, polysaccharides, chlorophylls, carotenoids, vitamins and minerals.

EXPERIMENTAL APPROACH: This work aims to study the impact of the 2 and 4 % microalga *Chlorella vulgaris* addition on the nutritional composition, bioactivity, structure and sensory profile of quark and cream cheese, both probiotic fermented products. Texture profile analysis and fundamental rheology measurements (oscillatory and stationary) were performed to evaluate the impact of *C. vulgaris* on the mechanical properties of the fresh cheese. The nutritional composition was evaluated using standard methods and bioactivity through the determination of total phenolic compounds and antioxidant capacity.¹

RESULTS AND CONCLUSIONS: *C. vulgaris* had an impact on the firmness of both cheeses. In general, the cheese with added *C. vulgaris* had a better nutritional profile, with an increase in protein content, content of Mg, P, S, Cu, Zn, Fe and Mn, and better bioactivity with an increase in the antioxidant activity. Sensory testing results were promising, especially for cream cheese.

NOVELTY AND SCIENTIFIC CONTRIBUTION: The enrichment of traditional foods such as fresh cheese with microalgae represents an interesting strategy to develop hybrid products (with protein from animal and vegetable sources), obtain innovative and more sustainable products, and improve their nutritional profile in terms of protein and mineral content and bioactivity.

Optimization of Heterotrophic Culture Conditions for the Algae *Graesiella emersonii* WBG-1 to Produce Proteins

Autor: Kaixuan Wang

Plants (Basel). 2023 Jun 9;12(12):2255. doi: 10.3390/plants12122255.

ABSTRACT

The aim of this study was to improve the protein content and yield of heterotrophic microalgal cultivation and establish a simple, economical, and efficient method for microalgal protein production using the novel green alga, *Graesiella emersonii* WBG-1, which has not been previously reported for heterotrophic cultivation. Through batch heterotrophic cultivation of this alga, we observed that glucose was the optimal carbon source, while it could not use sucrose as a carbon source. Biomass production and protein content were significantly reduced when sodium acetate was used as the carbon source. Compared with nitrate, protein content increased by 93% when urea was used as the nitrogen source. Cultivation temperature had a significant impact on biomass production and protein content. The optimal conditions were glucose as the carbon source at an initial concentration of 10 g/L, urea as the nitrogen source at an initial concentration of 1.62 g/L, and a culture temperature of 35 °C. On the second day of batch cultivation, the highest protein content (66.14%) was achieved, which was significantly higher than that reported in heterotrophic cultures of *Chlorella* and much higher than that reported for specially established technologies aimed at increasing the protein content, such as two-stage heterotrophic, heterotrophy-dilution-photoinduction, and mixotrophic processes. These results demonstrate the great potential of the heterotrophic cultivation of *G. emersonii* WBG-1 for protein production.

Potential Anti-Obesity, Anti-Steatosis, and Anti-Inflammatory Properties of Extracts from the Microalgae *Chlorella vulgaris* and *Chlorococcum amblyostomatis* under Different Growth Conditions

Autor: Ana Regueiras

Mar Drugs. 2021 Dec 22;20(1):9. doi: 10.3390/md20010009.

ABSTRACT



Microalgae are known as a producer of proteins and lipids, but also of valuable compounds for human health benefits (e.g., polyunsaturated fatty acids (PUFAs); minerals, vitamins, or other compounds). The overall objective of this research was to prospect novel products, such as nutraceuticals from microalgae, for application in human health, particularly for metabolic diseases. *Chlorella vulgaris* and *Chlorococcum amblyostomatis* were grown autotrophically, and *C. vulgaris* was additionally grown heterotrophically. Microalgae biomass was extracted using organic solvents (dichloromethane, ethanol, ethanol with ultrasound-assisted extraction). Those extracts were evaluated for their bioactivities, toxicity, and metabolite profile. Some of the extracts reduced the neutral lipid content using the zebrafish larvae fat metabolism assay, reduced lipid accumulation in fatty-acid-overloaded HepG2 liver cells, or decreased the LPS-induced inflammation reaction in RAW264.7 macrophages. Toxicity was not observed in the MTT assay in vitro or by the appearance of lethality or malformations in zebrafish larvae in vivo. Differences in metabolite profiles of microalgae extracts obtained by UPLC-LC-MS/MS and GNPS analyses revealed unique compounds in the active extracts, whose majority did not have a match in mass spectrometry databases and could be potentially novel compounds. In conclusion, microalgae extracts demonstrated anti-obesity, anti-steatosis, and anti-inflammatory activities and could be valuable resources for developing future nutraceuticals. In particular, the ultrasound-assisted ethanolic extract of the heterotrophic *C. vulgaris* significantly enhanced the anti-obesity activity and demonstrated that the alteration of culture conditions is a valuable approach to increase the production of high-value compounds.

Rapid Screening of Microalgae as Potential Sources of Natural Antioxidants

Autor: Na Wang

Foods. 2023 Jul 10;12(14):2652. doi: 10.3390/foods12142652.

ABSTRACT

In order to rapidly screen microalgae species as feedstocks for antioxidants, extracts were obtained from 16 microalgae strains (under 11 genera, 7 classes) using two methods: a one-step extraction with ethanol/water and a three-step fractionating procedure using hexane, ethylacetate, and water successively. Measuring the total phenol content (TPC), total carotenoid content (TCC), and antioxidant activity of the extracts, indicating TPC and TCC, played an important role in determining the antioxidant activity of the microalgae. A weighted scoring system was used to evaluate the antioxidant activity, and the scores of microalgal samples from two extraction methods were calculated using the same system. Among the investigated microalgae, *Euglena gracilis* SCSIO-46781 had the highest antioxidant score, contributing to high TPC and TCC, followed by *Arthrospira platensis* SCSIO-44012, *Nannochloropsis* sp. SCSIO-45224, *Phaeodactylum tricornutum* SCSIO-45120, and *Nannochloropsis* sp. SCSIO-45006, respectively. Additionally, the above-mentioned five strains are currently being applied in commercial production, indicating this system could be effective not only for screening microalgal antioxidants, but also for screening microalgal species/strains with strong adaptation to environmental stress, which is a critical trait for their commercial cultivation.

Recent Progress on Systems and Synthetic Biology of Diatoms for Improving Algal Productivity

Autor: Jiwei Chen

Front Bioeng Biotechnol. 2022 May 13;10:908804. doi: 10.3389/fbioe.2022.908804. eCollection 2022.

ABSTRACT

Microalgae have drawn much attention for their potential applications as a sustainable source for developing bioactive compounds, functional foods, feeds, and biofuels. Diatoms, as one major group of microalgae with high yields and strong adaptability to the environment, have shown advantages in developing photosynthetic cell factories to produce value-added compounds, including heterologous bioactive products. However, the commercialization of diatoms has encountered several obstacles that limit the potential mass production, such as the limitation of algal productivity and low photosynthetic efficiency. In recent years, systems and synthetic biology have dramatically improved the efficiency of diatom cell factories. In this review, we discussed first the genome sequencing and genome-scale metabolic models (GEMS) of diatoms. Then, approaches to optimizing



photosynthetic efficiency are introduced with a focus on the enhancement of biomass productivity in diatoms. We also reviewed genome engineering technologies, including CRISPR (clustered regularly interspaced short palindromic repeats) gene-editing to produce bioactive compounds in diatoms. Finally, we summarized the recent progress on the diatom cell factory for producing heterologous compounds through genome engineering to introduce foreign genes into host diatoms. This review also pinpointed the bottlenecks in algal engineering development and provided critical insights into the future direction of algal production.

Revolutionizing biofuel generation: Unleashing the power of CRISPR-Cas mediated gene editing of extremophiles

Autor: Diksha Garg

Microbiol Res. 2023 Sep;274:127443. doi: 10.1016/j.micres.2023.127443. Epub 2023 Jun 26.

ABSTRACT

Molecular biology techniques like gene editing have altered the specific genes in micro-organisms to increase their efficiency to produce biofuels. This review paper investigates the outcomes of Clustered regularly interspaced short palindromic repeats (CRISPR) for gene editing in extremophilic micro-organisms to produce biofuel. Commercial production of biofuel from lignocellulosic waste is limited due to various constraints. A potential strategy to enhance the capability of extremophiles to produce biofuel is gene-editing via CRISPR-Cas technology. The efficiency of intracellular enzymes like cellulase, hemicellulase in extremophilic bacteria, fungi and microalgae has been increased by alteration of genes associated with enzymatic activity and thermotolerance. extremophilic microbes like *Thermococcus kodakarensis*, *Thermotoga maritima*, *Thermus thermophilus*, *Pyrococcus furiosus* and *Sulfolobus* sp. are explored for biofuel production. The conversion of lignocellulosic biomass into biofuels involves pretreatment, hydrolysis and fermentation. The challenges like off-target effect associated with use of extremophiles for biofuel production is also addressed. The appropriate regulations are required to maximize effectiveness while minimizing off-target cleavage, as well as the total biosafety of this technique. The latest discovery of the CRISPR-Cas system should provide a new channel in the creation of microbial biorefineries through site- specific gene editing that might boost the generation of biofuels from extremophiles. Overall, this review study highlights the potential for genome editing methods to improve the potential of extremophiles to produce biofuel, opening the door to more effective and environmentally friendly biofuel production methods.

Safety of an ethanolic extract of the dried biomass of the microalga *Phaeodactylum tricornutum* as a novel food pursuant to Regulation (EU) 2015/2283

Autor: EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA)

EFSA J. 2023 Jul 12;21(7):e08072. doi: 10.2903/j.efsa.2023.8072. eCollection 2023 Jul.

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on an ethanolic extract of the dried biomass of the microalga *Phaeodactylum tricornutum* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is an ethanolic extract of the dried biomass of the microalga *P. tricornutum* diluted in a medium-chain triglyceride oil carrier, with standardised fucoxanthin and tocopherol content. The main component of the NF is fat (78% on average), followed by crude protein (10% on average). The Panel is of the view that a consistent and safe production process has not been demonstrated. Additionally, the Panel considers that the information provided on the composition of the NF is not complete and may raise safety concerns. The applicant proposed to use the NF as a food supplement at the use level of 437 mg/day, with the target population being adults, excluding pregnant and breastfeeding women. There is no history of use of the NF or of its source, i.e. *P. tricornutum*. The Panel notes that the source of the NF, *P. tricornutum*, was not granted the qualified presumption of safety (QPS) status by the EFSA Panel on Biological Hazards (BIOHAZ), due to the lack of a safe history of use in the food chain and



on its potential for production of bioactive compounds with toxic effects. There were no concerns regarding genotoxicity of the NF. In the 90-day study provided, a number of adverse effects were observed, some of them seen already at the lowest dose tested (750 mg/kg body weight (bw) day), which was identified by the Panel as the lowest-observed-adverse-effect-level (LOAEL). The potential phototoxicity of pheophorbide A and pyropheophorbide A in the NF was not addressed in this study. Although noting the uncertainties identified by the Panel regarding the analytical determination of these substances in the NF and the limitations in the publicly available toxicity data, a low margin of exposure (MoE) was calculated for these substances at the proposed use levels. The Panel concludes that the safety of the NF under the proposed uses and use levels has not been established.

Safety of paramylon as a novel food pursuant to Regulation (EU) 2015/2283

Autor: EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel)
EFSA J. 2023 May 26;21(5):e07995. doi: 10.2903/j.efsa.2023.7995. eCollection 2023 May.

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on paramylon as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Paramylon is a linear, unbranched beta-1,3-D-glucan polymer that is isolated from the single-cell microalga *Euglena gracilis*. The NF consists of at least 95% beta-glucan and minor amounts of protein, fat, ash and moisture. The applicant proposed to use the NF in food supplements, as a food ingredient added to a number of food categories and in foods for total diet replacement for weight control. In 2019, *E. gracilis* was attributed the qualified presumption of safety (QPS) status with the qualification 'for production purposes only', which includes food products based on microbial biomass of the microalga. Based on the information provided, *E. gracilis* is not expected to survive the manufacturing process. The submitted toxicity studies did not raise safety concerns. No adverse effects were observed in the subchronic toxicity studies, up to the highest dose tested, i.e. 5,000 mg NF/kg body weight per day. In view of the QPS status of the source of the NF, supported by the manufacturing process, compositional data and lack of toxicity observed in the toxicity studies, the Panel has no safety concerns and concludes that the NF, i.e. paramylon, is safe under the proposed uses and use levels.

Scenedesmus rubescens Heterotrophic Production Strategies for Added Value Biomass

Autor: Gonçalo Espírito Santo
Mar Drugs. 2023 Jul 19;21(7):411. doi: 10.3390/md21070411.

ABSTRACT

Microalgae attract interest worldwide due to their potential for several applications. *Scenedesmus* is one of the first in vitro cultured algae due to their rapid growth and handling easiness. Within this genus, cells exhibit a highly resistant wall and propagate both auto- and heterotrophically. The main goal of the present work is to find scalable ways to produce a highly concentrated biomass of *Scenedesmus rubescens* in heterotrophic conditions. *Scenedesmus rubescens* growth was improved at the lab-scale by 3.2-fold (from 4.1 to 13 g/L of dry weight) through medium optimization by response surface methodology. Afterwards, scale-up was evaluated in 7 L stirred-tank reactor under fed-batch operation. Then, the optimized medium resulted in an overall productivity of 8.63 g/L/day and a maximum biomass concentration of 69.5 g/L. *S. rubescens* protein content achieved approximately 31% of dry weight, similar to the protein content of *Chlorella vulgaris* in heterotrophy.

Simultaneous bioremediation of petroleum hydrocarbons and production of biofuels by the micro-green alga, cyanobacteria, and its consortium

Autor: Ragaa A Hamouda
Heliyon. 2023 Jun 5;9(6):e16656. doi: 10.1016/j.heliyon.2023.e16656. eCollection 2023 Jun.

ABSTRACT



There are two major problems in the world, fuel deficiency and environmental pollution by fossil fuels. Microalgae are regarded as one of the most feasible feedstocks for the manufacturing of biofuels and are used in the degradation of fossil fuel spills. The present study was possessed to investigate the ability of green alga *Chlorella vulgaris*, blue-green alga *Synechococcus* sp, and its consortium to grow and degrade hydrocarbon such as kerosene (k) with different concentrations (0, 0.5, 1, and 1.5%), and also using algal biomasses to produce biofuel. The algal growth was estimated by optical density (O.D) at 600 nm, pigment contents such as Chlorophyll a,b carotenoid, and dry weight. The kerosene degradation was estimated by FT-IR analysis after and before the cultivation of algae and its consortium. The components of the methanol extract were determined by GC-MS spectroscopy. The results denote the best growth was determined by O.D, algae consortium with 1.5% Kerosene after ten days, meanwhile, the highest dry weight was with *C. vulgaris* after ten days of cultivation. The FT-IR demonstrated the algae and consortium possessed high efficacy to degrade kerosene. After 15 days of algae cultivation with 1% K, *C.vulgaris* produced the maximum amount of lipids (32%). The GC-MS profile of methanol extract of two algae and consortium demonstrated that Undecane was presented in high amounts, *C.vulgaris* (19.9%), *Synechococcus* sp (82.16%), algae consortium (79.51%), and also were presented moderate amounts of fatty acid methyl ester in *Synechococcus* sp. Overall, our results indicate that a consortium of algae can absorb and remove kerosene from water, and at the same time produce biofuels like biodiesel and petroleum-based fuels.

Sustainable production and pharmaceutical applications of β -glucan from microbial sources

Autor: Emma J Murphy

Microbiol Res. 2023 Sep;274:127424. doi: 10.1016/j.micres.2023.127424. Epub 2023 Jun 5.

ABSTRACT

β -glucans are a large class of complex polysaccharides found in abundant sources. Our dietary sources of β -glucans are cereals that include oats and barley, and non-cereal sources can consist of mushrooms, microalgae, bacteria, and seaweeds. There is substantial clinical interest in β -glucans; as they can be used for a variety of diseases including cancer and cardiovascular conditions. Suitable sources of β -glucans for biopharmaceutical applications include bacteria, microalgae, mycelium, and yeast. Environmental factors including culture medium can influence the biomass and ultimately β -glucan content. Therefore, cultivation conditions for the above organisms can be controlled for sustainable enhanced production of β -glucans. This review discusses the various sources of β -glucans and their cultivation conditions that may be optimised to exploit sustainable production. Finally, this article discusses the immune-modulatory potential of β -glucans from these sources.

The Effect of Oil-Rich Food Waste Substrates, Used as an Alternative Carbon Source, on the Cultivation of Microalgae-A Pilot Study

Autor: Pavlína Sniegoňová

Microorganisms. 2023 Jun 21;11(7):1621. doi: 10.3390/microorganisms11071621.

ABSTRACT

Microalgae are mostly phototrophic microorganisms present worldwide, showcasing great adaptability to their environment. They are known for producing essential metabolites such as carotenoids, chlorophylls, sterols, lipids, and many more. This study discusses the possibility of the mixotrophic abilities of microalgae in the presence of food waste oils. The utilization of food waste materials is becoming more popular as a research subject as its production grows every year, increasing the environmental burden. In this work, waste frying oil and coffee oil were tested for the first time as a nutrition source for microalgae cultivation. Waste frying oil is produced in large amounts all over the world and its simple purification is one of its greatest advantages as it only needs to be filtered from leftover food pieces. Coffee oil is extracted from waste spent coffee grounds as a by-product. The waste frying oil and coffee oil were added to the basic algal media as an alternative source of carbon. As a pilot study for further experimentation, the effect of oil in the medium, algal adaptability, and capability to survive were tested within these experiments. The



growth and production characteristics of four algae and cyanobacteria strains were tested, of which the strain *Desmodesmus armatus* achieved exceptional results of chlorophyll (8.171 ± 0.475 mg/g) and ubiquinone (5.708 ± 0.138 mg/g) production. The strain *Chlamydomonas reinhardtii* showed exceptional lipid accumulation in the range of 30-46% in most of the samples.

The Metabolism of Reactive Oxygen Species and Their Effects on Lipid Biosynthesis of Microalgae

Autor: Liufu Wang

Int J Mol Sci. 2023 Jul 3;24(13):11041. doi: 10.3390/ijms241311041.

ABSTRACT

Microalgae have outstanding abilities to transform carbon dioxide (CO₂) into useful lipids, which makes them extremely promising as renewable sources for manufacturing beneficial compounds. However, during this process, reactive oxygen species (ROS) can be inevitably formed via electron transfers in basal metabolisms. While the excessive accumulation of ROS can have negative effects, it has been supported that proper accumulation of ROS is essential to these organisms. Recent studies have shown that ROS increases are closely related to total lipid in microalgae under stress conditions. However, the exact mechanism behind this phenomenon remains largely unknown. Therefore, this paper aims to introduce the production and elimination of ROS in microalgae. The roles of ROS in three different signaling pathways for lipid biosynthesis are then reviewed: receptor proteins and phosphatases, as well as redox-sensitive transcription factors. Moreover, the strategies and applications of ROS-induced lipid biosynthesis in microalgae are summarized. Finally, future perspectives in this emerging field are also mentioned, appealing to more researchers to further explore the relative mechanisms. This may contribute to improving lipid accumulation in microalgae.

The Microalgal Diatoxanthin Inflects the Cytokine Storm in SARS-CoV-2 Stimulated ACE2 Overexpressing Lung Cells

Autor: Clementina Sansone

Antioxidants (Basel). 2022 Aug 3;11(8):1515. doi: 10.3390/antiox11081515.

ABSTRACT

Contact between SARS-CoV-2 and human lung cells involves the viral spike protein and the human angiotensin-converting enzyme 2 (ACE2) receptor on epithelial cells, the latter being strongly involved in the regulation of inflammation as well as blood pressure homeostasis. SARS-CoV-2 infection is characterized by a strong inflammatory response defined as a "cytokine storm". Among recent therapeutic approaches against SARS-CoV-2 targeting the dramatic inflammatory reaction, some natural products are promising. Diatoms are microalgae able to produce bioactive secondary metabolites, such as the xanthophyll diatoxanthin (Dt). The aim of this study is to demonstrate the anti-inflammatory effects of Dt on the A549-hACE2 lung cell line, exploring its interaction with the ACE2 receptor, as well as depicting its role in inhibiting a cytokine storm induced by the SARS-CoV-2 spike glycoprotein. Results showed that Dt enhanced the cell metabolism, e.g., the percent of metabolically active cells, as well as the ACE2 enzymatic activity. Moreover, Dt strongly affected the response of the SARS-CoV-2 spike glycoprotein-exposed A549-hACE2 cells in decreasing the interleukin-6 production and increasing the interleukin-10 release. Moreover, Dt upregulated genes encoding for the interferon pathway related to antiviral defense and enhanced proteins belonging to the innate immunity response. The potential interest of Dt as a new therapeutic agent in the treatment and/or prevention of the severe inflammatory syndrome related to SARS-CoV-2 infection is postulated.

The Possibility of Deploying CO₂ from Biogas Combustion to Improve the Productivity of a Periodical *Chlorella vulgaris* Culture

Autor: Marcin Zieliński

Front Biosci (Elite Ed). 2023 Jan 16;15(1):3. doi: 10.31083/j.fbe1501003.



ABSTRACT

BACKGROUND: Carbon dioxide (CO₂) is the major contributor to the global emissions of greenhouse gases, which necessitates the search for its fixation and utilization methods. Engaging photosynthesizing microorganisms for its biosequestration is one of the prospective technologies applied to this end. Considering the paucity of literature works on the possibilities of deploying CO₂ from biogas combustion to intensify microalgae production, this research aimed to identify the feasibility of using this type of CO₂ in *Chlorella vulgaris* culture by evaluating biomass production yield and CO₂ biosequestration effectiveness.

METHODS: The experiment was performed in glass PBR, in which the culture medium occupied the volume of 1.0 dm³, and the gaseous phase occupied 0.3 dm³. The reactors were continuously illuminated by fluorescent lamps. The temperature of flue gases and air fed to reactors, and culture temperature was 20 °C ± 2 °C.

RESULTS: The use of flue gases promoted a more rapid biomass growth, reaching 77.8 ± 3.1 mgVS/dm³·d, and produced a higher microalgae concentration, i.e., 780 ± 58 mgVS/dm³. Nevertheless, the flue gas-fed culture turned out to be highly sensitive, which was manifested in a decreased culture medium pH and relatively quickly achieved decay phase of the *C. vulgaris* population. The microalgae effectively assimilated CO₂, reducing its concentration from 13 ± 1% to 1 ± 0.5% in the effluent from the photobioreactor.

CONCLUSIONS: The flue gases were found not to affect the qualitative composition of the microalgal biomass. However, strict control and monitoring of microalgae biomass production is necessary, as well as rapid responses in flue gas-fed systems. This is an important hint for potential operators of such technological systems on the large scale. Regardless of the possibility of deploying microalgae to fix and utilize CO₂, a justified avenue of research is to look for cheap sources of CO₂-rich gases.

The genomes of *Vischeria oleaginous* microalgae shed light on the molecular basis of hyper-accumulation of lipids

Autor: Baoyan Gao

BMC Biol. 2023 Jun 6;21(1):133. doi: 10.1186/s12915-023-01618-x.

ABSTRACT

BACKGROUND: With the urgent need to reduce carbon emissions, and the dwindling reserves of easily exploitable fossil fuel, microalgae-based biofuels that can be used for transport systems and CO₂ abatement have attracted great attention worldwide in recent years. One useful characteristic of microalgae is their ability to accumulate high levels of lipid content, in particular under conditions of nitrogen deprivation, with numerous species identified so far. However, a trade-off between levels of lipid accumulation and biomass productivity hinders the commercial applicability of lipids from microalgae. Here, we sequenced the genomes of *Vischeria* sp. CAUP H4302 and *Vischeria stellata* SAG 33.83, which can accumulate high content of lipids rich in nutraceutical fatty acids and with excellent biomass yield in nitrogen-limiting culture.

RESULTS: A whole-genome duplication (WGD) event was revealed in *V. sp. CAUP H4302*, which is a rare event in unicellular microalgae. Comparative genomic analyses showed that a battery of genes encoding pivotal enzymes involved in fatty acids and triacylglycerol biosynthesis, storage polysaccharide hydrolysis, and nitrogen and amino acid-related metabolisms are expanded in the genus *Vischeria* or only in *V. sp. CAUP H4302*. The most highlighted is the expansion of cyanate lyase genes in the genus *Vischeria*, which may enhance their detoxification ability against the toxic cyanate by decomposing cyanate to NH₃ and CO₂, especially under nitrogen-limiting conditions, resulting in better growth performance and sustained accumulation of biomass under the aforementioned stress conditions.

CONCLUSIONS: This study presents a WGD event in microalgae, providing new insights into the genetic and regulatory mechanism underpinning hyper-accumulation of lipids and offering potentially valuable targets for future improvements in oleaginous microalgae by metabolic engineering.

Towards microalga-based superfoods: heterologous expression of zeolin in *Chlamydomonas reinhardtii*

Autor: Federico Perozeni



ABSTRACT

Microalgae are unicellular photosynthetic organisms that can be grown in artificial systems to capture CO₂, release oxygen, use nitrogen- and phosphorus-rich wastes, and produce biomass and bioproducts of interest including edible biomass for space exploration. In the present study, we report a metabolic engineering strategy for the green alga *Chlamydomonas reinhardtii* to produce high-value proteins for nutritional purposes. *Chlamydomonas reinhardtii* is a species approved by the U.S. Food and Drug Administration (FDA) for human consumption, and its consumption has been reported to improve gastrointestinal health in both murine models and humans. By utilizing the biotechnological tools available for this green alga, we introduced a synthetic gene encoding a chimeric protein, zeolin, obtained by merging the γ -zein and phaseolin proteins, in the algal genome. Zein and phaseolin are major seed storage proteins of maize (*Zea mays*) and bean (*Phaseolus vulgaris*) that accumulate in the endoplasmic reticulum (ER) and storage vacuoles, respectively. Seed storage proteins have unbalanced amino acid content, and for this reason, need to be complemented with each other in the diet. The chimeric recombinant zeolin protein represents an amino acid storage strategy with a balanced amino acid profile. Zeolin protein was thus efficiently expressed in *Chlamydomonas reinhardtii*; thus, we obtained strains that accumulate this recombinant protein in the endoplasmic reticulum, reaching a concentration up to 5.5 fg cell⁻¹, or secrete it in the growth medium, with a titer value up to 82 μ g/L, enabling the production of microalga-based super-food.

Transcriptional insights into *Chlorella* sp. ABC-001: a comparative study of carbon fixation and lipid synthesis under different CO₂ conditions

Autor: Hyun Gi Koh

Biotechnol Biofuels Bioprod. 2023 Jul 15;16(1):113. doi: 10.1186/s13068-023-02358-4.

ABSTRACT

BACKGROUND: Microalgae's low tolerance to high CO₂ concentrations presents a significant challenge for its industrial application, especially when considering the utilization of industrial exhaust gas streams with high CO₂ content—an economically and environmentally attractive option. Therefore, the objectives of this study were to investigate the metabolic changes in carbon fixation and lipid accumulation of microalgae under ambient air and high CO₂ conditions, deepen our understanding of the molecular mechanisms driving these processes, and identify potential target genes for metabolic engineering in microalgae. To accomplish these goals, we conducted a transcriptomic analysis of the high CO₂-tolerant strain, *Chlorella* sp. ABC-001, under two different carbon dioxide levels (ambient air and 10% CO₂) and at various growth phases.

RESULTS: Cells cultivated with 10% CO₂ exhibited significantly better growth and lipid accumulation rates, achieving up to 2.5-fold higher cell density and twice the lipid content by day 7. To understand the relationship between CO₂ concentrations and phenotypes, transcriptomic analysis was conducted across different CO₂ conditions and growth phases. According to the analysis of differentially expressed genes and gene ontology, *Chlorella* sp. ABC-001 exhibited the development of chloroplast organelles during the early exponential phase under high CO₂ conditions, resulting in improved CO₂ fixation and enhanced photosynthesis. Cobalamin-independent methionine synthase expression was also significantly elevated during the early growth stage, likely contributing to the methionine supply required for various metabolic activities and active proliferation. Conversely, the cells showed sustained repression of carbonic anhydrase and ferredoxin hydrogenase, involved in the carbon concentrating mechanism, throughout the cultivation period under high CO₂ conditions. This study also delved into the transcriptomic profiles in the Calvin cycle, nitrogen reductase, and lipid synthesis. Particularly, *Chlorella* sp. ABC-001 showed high expression levels of genes involved in lipid synthesis, such as glycerol-3-phosphate dehydrogenase and phospholipid-diacylglycerol acyltransferase. These findings suggest potential targets for metabolic engineering aimed at enhancing lipid production in microalgae.

CONCLUSIONS: We expect that our findings will help understand the carbon concentrating mechanism, photosynthesis, nitrogen assimilation, and lipid accumulation metabolisms of green algae according to CO₂ concentrations. This study also provides insights into systems metabolic engineering of microalgae for improved performance in the future.



Two-phase microalgae cultivation for RAS water remediation and high-value biomass production

Autor: Valeria Villanova

Front Plant Sci. 2023 Jun 12;14:1186537. doi: 10.3389/fpls.2023.1186537. eCollection 2023.

ABSTRACT

The overall goal of this study was to provide solutions to innovative microalgae-based technology for wastewater remediation in a cold-water recirculating marine aquaculture system (RAS). This is based on the novel concept of integrated aquaculture systems in which fish nutrient-rich rearing water will be used for microalgae cultivation. The produced biomass can be used as fish feed, while the cleaned water can be reused, to create a highly eco-sustainable circular economy. Here, we tested three microalgae species *Nannochloropsis granulata* (Ng), *Phaeodactylum tricornutum* (Pt), and *Chlorella* sp (Csp) for their ability to remove nitrogen and phosphate from the RAS wastewater and simultaneously produce high-value biomass, i.e., containing amino acids (AA), carotenoids, and polyunsaturated fatty acids (PUFAs). A high yield and value of biomass were achieved for all species in a two-phase cultivation strategy: i) a first phase using a medium optimized for best growth (f/2 14x, control); ii) a second "stress" phase using the RAS wastewater to enhance the production of high-value metabolites. Ng and Pt performed best in terms of biomass yield (i.e., 5-6 g of dry weight, DW.L-1) and efficient cleaning of the RAS wastewater from nitrite, nitrate, and phosphate (i.e., 100% removal). Csp produced about 3 g L-1 of DW and reduced efficiently only nitrate, and phosphate (i.e., about 76% and 100% removal, respectively). The biomass of all strains was rich in protein (30-40 % of DW) containing all the essential AA except Methionine. The biomass of all three species was also rich in PUFAs. Finally, all tested species are excellent sources of antioxidant carotenoids, including fucoxanthin (Pt), lutein (Ng and Csp) and β -carotene (Csp). All tested species in our novel two-phase cultivation strategy thus showed great potential to treat marine RAS wastewater and provide sustainable alternatives to animal and plant proteins with extra added values.

Use of a commercial feed supplement based on yeast products and microalgae with or without nucleotide addition in calves

Autor: Aristide Maggiolino

J Dairy Sci. 2023 Jun;106(6):4397-4412. doi: 10.3168/jds.2022-22656. Epub 2023 Apr 18.

ABSTRACT

The use of feed additives with antioxidant and immune response modulatory activity could be a useful strategy in suckling calves to reduce morbidity and mortality. This strategy is based on several feed additives tested for these purposes. The aim of the paper is the examination of a commercial feed additive for adult cows for use in calves, with and without nucleotide supplementation. Seventy-five Holstein Friesian male calves were divided in 3 groups, with each calf randomly assigned to a group according to birth order. All calves received 2 L of pooled colostrum within 2 h of birth. The commercial feed supplement group was orally administered with 5 g/head of Decosel (dried brewer's yeast lysate (*Saccharomyces cerevisiae*), brewer's yeast walls (*Saccharomyces cerevisiae*), diatoms, spirulina, barley flour, calcium carbonate; Agroteam srl, Torrimpietra, Italy) and the nucleotides + commercial feed supplement group was orally administered with 5 g/head of an additive containing 2.5 g of Decosel and 2.5 g of nucleotides once daily from birth to 25 d. The control group was orally administered 20 mL of fresh water/head once daily. Calves that received the supplement and the nucleotides showed lower rates of protein and metabolizable energy conversion, with longer villi and greater crypt depth in duodenum. Moreover, the commercial feed supplement alone increased antioxidant capacity [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and ferric-reducing antioxidant power] in plasma some activity of antioxidant liver enzymes, and peripheral blood mononuclear cell viability after in vitro concanavalin A and H₂O₂ stimuli. Dietary supplementation with a commercial feed supplement containing yeast products (yeast cell walls and hydrolyzed yeast) and microalgae enhanced the redox balance and gut morphology in calves, allowing calves to improve their immune response, increasing



resistance to stress. Moreover, these beneficial effects were strongly potentiated when dietary nucleotides were added to the supplement.

Weak acids produced during anaerobic respiration suppress both photosynthesis and aerobic respiration

Autor: Xiaojie Pang

Nat Commun. 2023 Jul 14;14(1):4207. doi: 10.1038/s41467-023-39898-0.

ABSTRACT

While photosynthesis transforms sunlight energy into sugar, aerobic and anaerobic respiration (fermentation) catabolizes sugars to fuel cellular activities. These processes take place within one cell across several compartments, however it remains largely unexplored how they interact with one another. Here we report that the weak acids produced during fermentation down-regulate both photosynthesis and aerobic respiration. This effect is mechanistically explained with an "ion trapping" model, in which the lipid bilayer selectively traps protons that effectively acidify subcellular compartments with smaller buffer capacities - such as the thylakoid lumen. Physiologically, we propose that under certain conditions, e.g., dim light at dawn, tuning down the photosynthetic light reaction could mitigate the pressure on its electron transport chains, while suppression of respiration could accelerate the net oxygen evolution, thus speeding up the recovery from hypoxia. Since we show that this effect is conserved across photosynthetic phyla, these results indicate that fermentation metabolites exert widespread feedback control over photosynthesis and aerobic respiration. This likely allows algae to better cope with changing environmental conditions.



PATENTES

BIO-OIL EXTRACTION METHOD WITH IMPROVED OIL RECOVERY RATE BY USING COOLING PROCESS

Inventor(s): JEONG A YOUNG [KR]; AN JUNGAP [KR]; LEE IN [KR] +
Applicant(s): CJ CHEILJEDANG CORP [KR] +

The present invention relates to a bio-oil extraction method with an improved oil recovery rate by using a cooling process. The bio-oil extraction method of the present invention, in which a process of cooling a suspension obtained by disrupting cells of a culture solution containing microalgae is further carried out, exhibits a high oil recovery rate, and does not comprise adding other additives to increase an oil recovery rate, thus enabling oil recovery and the use of remaining by-products as feed, whereby sustainable production is possible, and the recovered oil can be effectively used as a feed composition, a food composition, or the like.

PRODUCTION OF MICROALGAE WITH HIGH LIPID PRODUCTIVITY

Inventor(s): POLAT ECE [TR]; ALTINBAS MAHMUT [TR] +
Applicant(s): UNIV ISTANBUL TEKNIK [TR] +

The present invention relates to the development of strains of microalgae that show high lipid productivity, particularly suitable for the production of biofuels, the growth of related microalgae and microalgae suitable for biofuel production. With the invention, a method has been developed that allows the development of strains compatible with magnesium starvation by fed-batch culture transfer of *Auxenochlorella protothecoides* microalgae. Also, with the invention, a strain of *Auxenochlorella protothecoides* has also been developed that is suitable for biofuel production and shows high lipid productivity.

METHODS AND FORMULATIONS FOR ENHANCING HIGH VALUE LIPIDS

Inventor(s): MATHUR ANSHU SHANKAR [IN]; MEHTA PREETI [IN]; RANI REKHA [IN]; GUPTA RAVI PRAKASH [IN]; PURI SURESH KUMAR [IN]; RAMAKUMAR SANKARA SRI VENKATA [IN] +
Applicant(s): INDIAN OIL CORP LTD [IN]; DEPT OF BIOTECHNOLOGY INDIA [IN] +

The present invention provides a method for the simultaneous enhancement in biomass and lipids containing omega-3-fatty acids of Thraustochytrid microalgae in a single step using synergistic effect of chemical mixture in appropriate proportion in production medium. The process discloses enriching the biomass of microalgae with high value lipids by subjecting the microalgal cells in growth medium supplemented with unique combination of chemical modulators and carbon substrates in the presence of nitrogen. The present invention also provides a novel strain *Schizochytrium* sp. (MTCC 5980) for use in continuous aerobic fermentative lipid production process for enhancing high value lipids like Docosahexaenoic acid (DHA), Docasapentaenoic acid (DPA), Eicosapentaenic acid (EPA) and lipids for biodiesel.

VERTICAL CONTINUOUS MULTIPHASE REACTOR FOR THE CLEAN PRODUCTION OF HYDROCARBONS AND ENERGY AND THERMOCHEMICAL METHOD CARRIED OUT

Inventor(s): GUTIERREZ FONSECA JAIME EDUARDO [CO] +
Applicant(s): BIOTECNOLOGIA Y BIOINGENIERIA COE S A [CO] +



Disclosed is a reactor and method for thermochemically degrading wet biomass without the need for prior drying, in particular microalga-rich substrates. The invention provides a vertical continuous multiphase reactor (VCMR) that simultaneously, progressively and continuously carries out the steps of evaporation, pyrolysis, gasification and combustion, in separate chambers, using indirect heating. The reactor operates at pressures below atmospheric pressure to increase thermal and productive efficiency, using a fraction of the same products as fuel to achieve thermal self-sufficiency. A system for instant evaporation at low temperature by means of adiabatic expansion is used. The reactor has high efficiency and high yield, requiring minimum space, and can be movable. The products obtained from the reactor are synthesis gas, biocarbon and bio-oils, with uses in energy, agriculture, cosmetics, health and construction. The invention also provides a method for obtaining hydrocarbons and energy from high-moisture biomass, wherein the steps are carried out continuously and the method does not need to be interrupted to add new wet biomass for conversion.

MODULAR OPEN TYPE MICROALGAE CULTIVATION PONDS SYSTEM

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Applicant(s): RĪGAS TEHNISKĀ UNIVERSITĀTE [LV] +

The invention relates to the fishing industry or the biogas sector, in particular to microalgae cultivation technologies for obtaining biomass. The invention is a modular open raceway pyramidally arranged with an increased light transmission for a microalgae cultivation system. The use of a transparent material for the production of open-type microalgae cultivation ponds (1) provides greater light access to the microalgae cultures, ensuring higher microalgae productivity. Pyramidally-structured cultivation ponds (1) provide 33 % land savings and 60 % more natural light access compared to the one level cultivation ponds. The invention is intended for the cultivation of microalgae with high biomass productivity, which can be further used as a raw material for the production of bioenergy or high-value products. The cultivation of microalgae can be carried out using wastewaters (e.g. liquid digestate from biogas plant) and flue gases as source of nutrients and carbon for microalgae, which will also result in wastewater treatment and reduction of CO₂ emissions.

PROCESS FOR PRODUCTION OF ENRICHED ALGAL BIOMASS

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Applicant(s): INDIAN OIL CORP LTD [IN]; DEPT OF BIOTECHNOLOGY INDIA [IN] +

The present invention relates to a process for producing enriched algal biomass having high lipid productivity. More particularly, the present invention provides a process for obtaining an enriched biomass with omega-3 fatty acids by using a microalgal strain *Schizochytrium* MTCC 5980 in a unique media composition and substrate residual band in a steady state continuous fermentation. The process of the present invention results in high biomass and lipid productivity.

Productivity and Bioproduct Formation in Phototropin Knock/Out Mutants in Microalgae

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Applicant(s): NMC INC [US]; LOS ALAMOS NAT SECURITY LLC [US] +



Phototropin is a blue light receptor, which mediates a variety of blue-light elicited physiological processes in plants and algae. In higher plants these processes include phototropism, chloroplast movement and stomatal opening. In the green alga *Chlamydomonas reinhardtii*, phototropin plays a vital role in progression of the sexual life cycle and in the control of the eye spot size and light sensitivity. Phototropin is also involved in blue-light mediated changes in the synthesis of chlorophylls, carotenoids, chlorophyll binding proteins. We compared the transcriptome of phototropin knock out (PHOT KO) mutant and wild-type parent to analyze differences in gene expression in high light grown cultures (500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). Our results indicate the up-regulation of genes involved in photosynthetic electron transport chain, carbon fixation pathway, starch, lipid, and cell cycle control genes. With respect to photosynthetic electron transport genes, genes encoding proteins of the cytochrome b6f and ATP synthase complex were up regulated potentially facilitating proton-coupled electron transfer. In addition genes involved in limiting steps in the Calvin cycle Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), Sedoheptulose 1,7 bisphosphatase (SBPase), Glyceraldehyde-3-phosphate dehydrogenase (3PGDH) and that mediate cell-cycle control (CDK) were also up regulated along with starch synthase and fatty acid biosynthesis genes involved in starch and lipid synthesis. In addition, transmission electron micrographs show increased accumulation of starch granules in PHOT mutant compared to wild type, which is consistent with the higher expression of starch synthase genes. Collectively, the altered patterns of gene expression in the PHOT mutants were associated with a two-fold increase in growth and biomass accumulation compared to wild type when grown in environmental photobioreactors (Phenometrics) that simulate a pond environment. In conclusion, our studies suggest that phototropin may be a master gene regulator that suppresses rapid cell growth and promotes gametogenesis and sexual recombination in wild type strains.

BIOTECHNOLOGICAL PROCESS FOR STIMULATION OF GROWTH OF CHLORELLA SOROKINIANA UTEX 1230 BIOMASS QUANTITY

Inventor(s): ARDELEAN IOAN [RO]; MOISESCU CRISTINA [RO]; ARDELEAN ANA VALENTINA [RO]; NEGUJ CONSTANTIN DANIEL [RO] +
Applicant(s): INST DE BIOLOGIE BUCURESTI AL ACADEMIEI ROMANE [RO] +

The invention relates to a biotechnological process for obtaining *Chlorella sorokiniana* UTEX 1230 green microalgae biomass. According to the invention, the process consists in that the biomass of *Chlorella sorokiniana* UTEX 1230 microalgae strain cultivated in a medium known per se, in the exponential growth phase, having a optical density (OD) of about 0.05 OD units at 660 nm, is irradiated with γ rays of 75 Gy, at a dose flow rate of 0.1...1.0 Gy/s, for 1...10 min, until the algal culture reaches, by growth and cell multiplication, about 0.2 OD units at 660 nm, the so irradiated culture being admixed with a 150...230 times higher amount of non-irradiated culture medium and maintained for 7 days at atmospheric pressure, at a temperature of 30°C and illumination of 16500 lux, in a medium continuously bubbled with atmospheric air, to result in an increase of harvested biomass yield, of total proteins and lipids, of total carotenes and chlorophyll.

BIOPOWERPLANT: THIRD GENERATION BIOREFINERY WITH IMPROVED CAPACITY TO USE DOMESTIC WASTEWATER, LANDFILL LEACHATE AND SEA SALT WATER AS AN INPUT TO GENERATE GREEN ENERGY, WATER FOR REUSE, BIOFUEL, ORGANIC FERTILIZERS AND CAPTURE ATMOSPHERIC CO₂

Inventor(s): GUTIÉRREZ FONSECA JAIME EDUARDO [CO] +
Applicant(s): DETEHC LLC [US] +

The Biopowerplant is a system that integrates the generation of carbon-neutral energy through the cultivation and conversion of microalgal biomass, with sewage sanitation and environmental carbon recovery, with the additional and secondary production of biofertilizer, biofuel, water for reuse. This system integrates a suboptimal anaerobic



digestion subsystem focused on the generation of biogas, the processing of the resulting digestate through a microalgal consortium culture subsystem with biofilm induction and smooth decreasing gradient of light radiation, and the transformation of the generated microalgal biomass into syngas through a subsystem of evaporation, torrefaction, pyrolysis, gasification, and combustion in separate chambers. The syngas and methane from the biogas are subsequently used as fuel in an electric power generator capable of operating with mixed gases. The biogas generation process is enriched through the recirculation of the microalgal biomass supernatant, the residual heat from the syngas generation subsystem, and the heat transferred from the combustion gases of the electric generator. The residual sludge from the biogas generation subsystem is recirculated towards a longitudinal biopile subsystem, where it acts as an anaerobic medium compared to the aerobic medium that constitutes the concentrated microalgal biomass, and both streams are mixed to be transformed into the syngas generation subsystem. Input inflows for system operation are mainly sewage, and optionally seawater and/or leachate. The inflows must be bioaugmented with a microalgal consortium dosed automatically by a Compact in situ bioaugmentation system, preferably more than 3 kilometers before the inflow enters the system.

Microalgae carbon sequestration and hydrogen production integrated coupling system

Inventor(s): HUANG JIANKE; LU JIAYING; DING RUOWEN; GUO ZIRU; LI JIAYANG; WANG JUNWEI; YU-WANG GUANGMING; WEI LIANG +
Applicant(s): UNIV HOHAI +

The utility model relates to a microalgae carbon sequestration and hydrogen production integrated coupling system, the coupling system comprises a main body structure and an auxiliary tank, the main body structure comprises three parts of an algae culture tank, an oxygen elimination tank and a hydrogen production tank which are longitudinally arranged, and the three parts are connected in series through valves in sequence from top to bottom; the auxiliary tank comprises a microalgae culture medium storage tank, a Na₂SO₃ mother liquor storage tank, a hydrogen temporary storage tank, a liquid CO₂ storage tank and a liquid pump. The culture medium storage tank is communicated with the top of the algae culture tank through a pipeline with a liquid pump, the liquid CO₂ storage tank is communicated with the top of the algae culture tank through a pipeline, the bottom of the Na₂SO₃ mother liquor storage tank is communicated with the top of the hydrogen production tank through a pipeline, and the top of the hydrogen temporary storage tank is communicated with the hydrogen production tank through a pipeline. The structure is integrally designed, modularized in combination, convenient to transport, flexible to use, low in system operation energy consumption, high in hydrogen production efficiency and suitable for being used as an integrated system for microalgae carbon sequestration and hydrogen production.

Method for producing biodiesel by using residual sludge to culture microalgae

Inventor(s): REN HONGYU; SONG XUETING; LIU BINGFENG; KONG FANYING; SONG QINGQING; REN NANQI +
Applicant(s): HARBIN INST TECHNOLOGY +

The invention relates to a method for producing biodiesel by culturing microalgae with excess sludge, belongs to the technical field of microalgae biology, and particularly relates to a method for producing biodiesel by culturing microalgae with excess sludge. The invention aims to solve the technical problem of low utilization rate of residual sludge at present. The residual sludge is pretreated, a high-pressure homogenizer is used for high-pressure crushing, supernatant is taken through centrifugation to serve as a culture medium for microalgae growth and grease production, and then a combined process of waste utilization and microalgae oil production is established. According to the method disclosed by the invention, resource reutilization of residual sludge waste at different temperatures is realized, meanwhile, efficient accumulation of microalgae grease is promoted, the cost of producing biodiesel by microalgae is reduced, and the method is



particularly suitable for microalgae biodiesel production in cold regions in northern China. The method is used for producing the biodiesel.

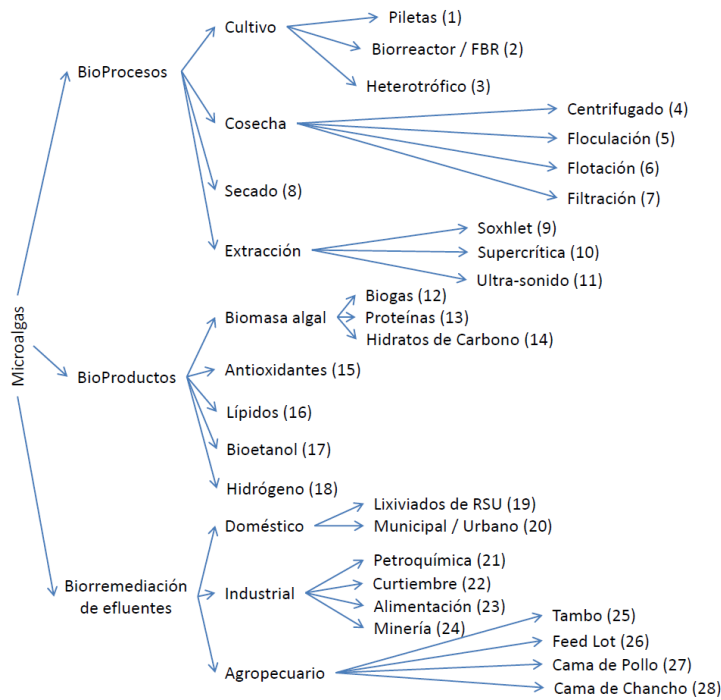
Method for improving yield of microalgae lipid by using recycled wastewater

Inventor(s): YU XUYA; SHI ZHONGDI; GU DAN +
Applicant(s): UNIV KUNMING SCIENCE & TECH +

The invention belongs to the technical field of bioengineering, and discloses a method for improving the yield of microalgae lipid by using recycled wastewater, which adopts a two-stage culture mode, and comprises the following steps: carrying out algae seed mixotrophic culture in the first stage to enable algae cells to rapidly grow in a short time, then collecting wastewater after microalgae culture, simply filtering, and proportioning BG-11 to form a mixed culture medium; and culturing the microalgae by using a mixed culture medium in combination with a photobioreactor. According to the method disclosed by the invention, a mixotrophic-photoautotrophic two-stage culture mode is combined, and in the photoautotrophic culture stage, when the concentration of the recycled wastewater is 40%, the lipid content and the lipid yield of the microalgae are respectively increased by 14.95% and 14.00% compared with those of a single BG-11 group; through the culture method disclosed by the invention, the lipid content and the lipid yield of the microalgae can be remarkably improved, rapid synthesis of the lipid in the microalgae cells is realized, the use of fresh water and the cost of microalgae culture are reduced, and a new research direction is provided for culturing the microalgae by using wastewater and treating the recycling cost.



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