Microalgal communities for green transition

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Original scientific article
Izvirni znanstveni članek

Abstract
Our world is approaching a critical juncture in which key resources for life on the planet (energy, nutrients, water and soil) are becoming ever more subject to climate and other environmental pressures. Algal–bacterial communities will likely have important ecological significance for the future, as well as relevance for the algal biotechnology processes. They can be used for a variety of products e.g. food, feed, fertilizers, pigments, bioplastics, biodiesel, biogas, bioethanol, bio-hydrogen and bio-stimulants. They can be involved in wastewater treatment and reuse. Evidence on symbiotic communities of algal-bacterial comunities points to the role of small (micro- and nano-sized) cellular particles (SCPs) shed by microorganisms and cells (extracellular vesicles, lipoproteins, antibody complexes, viruses) in their organization and evolution as a community. Combination of SCPs of natural origin and liposomes would circumvent robust cellular manipulation needed for isolation of natural SCPs which is expensive and time consuming and enable artificial nanocarriers to entrap biologically active molecules e.g. bio-stimulants. Employment of this nanotechnological strategy could generate efficient and reproducible biocompatible carriers for downstream applications.

Mikroalgne združbe za zeleni prehod

Povzetek
Naš svet se približuje kritični točki, v kateri postajajo ključni viri za življenje na planetu (energija, hranila, voda, tla) vse bolj podvrženi podnebnim in drugim okoljskim pritiskom. Združbe alg in bakterij bodo verjetno imele pomemben ekološki pomen za prihodnost, zlasti v biotehnoloških postopkih alg. Iz alg lahko pridobivamo različne produkte kot na primer hrano, krmo, gnojila, pigmente, bioplastiko, biodizel, bioplin, bioetanol, biovodik in biostimulanse. Lahko se jih uporabi za čiščenje in ponovno uporabo odpadne vode. Dokazi o simbiotskih združbah alg in bakterij kažejo na pomembno vlogo majhnih (mikro in nano velikih) celičnih delcev (SCP-jev), ki jih izločajo mikroorganizmi in celice (zunajceličnih veziklov, lipoproteinov, protitelesnih kompleksov, virusov) pri organizaciji in razvoju združb mikroorganizmov. Kombinacija SCP-jev naravnega izvora in liposomov bi se izognila robustni celični manipulaciji, potrebni za izolacijo naravnih SCP-jev, ki je draga in dolgotrajna, in bi omogočila umetnim nano nosilcem, da ujamejo biološko aktivne molekule kot so npr. biostimulansi. Uporaba take nanotehnološke strategije bi lahko ustvarila učinkovite in ponovljive biokompatibilne nosilce za nadaljnje aplikacije.

UNSEEN MAJORITY

Our world is approaching a critical juncture in which key resources for life on the planet (energy, nutrients, water and soil) are becoming even more a subject to climate and other environmental pressures. While the European Commission has specified nature-based solutions as likely means for understanding and managing the urban ecosystem's equilibrium, there is also rising awareness that the responses of microorganisms are essential for achieving an environmentally sustainable future (Cavicchioli et al., 2019, Atanasova et al., 2021). To assess this urgent need, nature-based solutions should be approached at the microscopic level, to uncover the natural laws governing biosphere processes involving complex communities composed of the 'unseen majority' – the microorganisms that carry out much of the production, consumption, decomposition, and nutrient cycling within ecosystems (Zimmerman et al., 2014). Algae contribute approximately half of the global net primary production (Field et al., 1998) and with bacterioplankton dominate the aquatic ecosystem (Sarmento and Gasol, 2012), influence the global carbon cycle and ultimately the whole climate. Algal–bacterial modes of interactions will likely have enormous ecological significance for the future, as well as relevance for algal biotechnologies (Ramanan et al., 2016). Furthermore, algae are known to produce a variety of compounds *e.g.* food, feed, fertilizers, pigments, bio-plastics, biodiesel, biogas, bioethanol, bio-hydrogen and bio-stimulants. They can be involved in wastewater treatment and reuse. Moreover, these systems have remediation abilities through reduction, stabilization, sequestration, assimilation, detoxification, mineralization, and decomposition, and provide for a high level of biodiversity, stability and flexibility of ecosystems. They do not compete with food or other crops as they can be cultivated on arid and unhospitable land and can be harvested on large commercial scale in aqueous suspensions where sufficient light and carbon dioxide is available.

Nature-based solutions for wastewater treatment with their ability for integrated resource management are promising for developing a circular economy in the urban environment (Langergraber et al., 2021). One such example is algal photobioreactors, most notably, high-rate algal ponds (HRAP), which rely on algae and bacterial communities to treat wastewater and produce biomass (Norvill et al., 2016). Compared to activated sludge reactors used for treating wastewater, HRAPs have longer hydraulic retention times and a large surface area, i.e., 1 ha or more in full-size HRAP systems (Norvill et al., 2016, Craggs et al., 2012, Sutherland et al., 2020). In addition, they do not require active aeration since the algae produce O_2 and organic acids needed by the bacteria, which contribute CO_2 and nutrients for the algae (Norvill et al., 2016). The main advantage of photobioreactors is the production of nutrient- and energy-rich algal biomass that may be exploited in a variety of products (Brennan and Owende, 2010; Park et al., 2011; Rahman et al., 2017). However, our understanding of nature-based solutions needs to progress from the technological unit level to the cellular community level since cellular communication plays a fundamental role in the homeostasis of complex biological systems where synchronization, cooperation, quick adaptation and specialization/differentiation of the cells occurs (Armingol et al., 2021; Combarnous et al., 2020; Jin et al., 2021).

ALGAE ADDRESS THE PROBLEM OF CONTAMINANTS OF EMERGING CONCERN

Contaminants of emerging concern (CECs) include active components of human and veterinary pharmaceuticals, illicit drugs, personal care products, pesticides, hormones, flame retardants, plasticizers and other compounds, as well as their metabolites and transformation products (TPs) (Noguera-Oviedo et al., 2016; Tolboom et al., 2019). However, their environmental occurrence and fate have been investigated only recently due to awareness of potential adverse ecological and human health impacts, although CECs may not be new in the environment (Yadav et al., 2021). CECs are typically present in the environment at trace levels, and only recent advances in analytical instrumentation have allowed their detection at low concentrations (ng/L and even pg/L) (Noguera-Oviedo et al., 2016). Bisphenols (BPs) are a group of CECs characterized by two hydroxyphenyl groups bound by a hydrocarbon bridge and otherwise containing diverse chemical groups, resulting in different physicochemical properties and consequent environmental behavior, making them suitable model compounds. BPs are monomers used to produce polycarbonate, epoxy resin, polysulfone, polyacrylate, polyetherimide, and as an additive in thermal paper, polyvinyl chloride and other products (Geens et al.,2011), and it was indicated that their emissions into wastewater are not negligible (Vehar et al., 2021). Studies point toward BPs causing endocrine disruption and other toxic effects (e.g., reproductive toxicity), neurotoxicity and cytotoxicity which is concerning as they may cause ecological harm (Munoz et al., 2009). Wastewater represents the main influx of CECs to the environment due to inadequate removal during conventional wastewater treatment (Norvill et al., 2016; Tolboom et al., 2019; Sutherland et al., 2019). They may also pose a risk to humans when considering reusing treated wastewater products (e.g., reclaimed water and biomass) for activities such as agriculture.

Microalgae photobioreactors are an alternative to conventional wastewater treatment (Škufca et al., 2021). Biodegradation of CECs in microalgal photobioreactors results from the metabolism of microalgae and bacteria, either intracellularly or extracellularly (Maryjoseph et al., 2020). Co-metabolic biodegradation may be accomplished by non-specific enzymes produced to assimilate other organic compounds (Liu et al., 2021). Furthermore, microalgae often grow in co-culture with bacteria (Božič et al., 2021). Biodegradation of CECs may take place according to three scenarios: (1) microalgae do not directly degrade the compound but provide a favorable environment for bacteria, promoting biodegradation, (2) bacteria and microalgae both significantly and directly contribute to the biodegradation of the CEC and (3) microalgae and bacteria synergistically degrade the CEC, where one can degrade the intermediate products of the other or vice versa (Liu et al., 2021). Past experimental studies have shown that a co-culture of microalgae and bacteria is more efficient at removing organic pollutants than a single culture (Ji et al., 2018, Prosenc et al., 2021). Some bacterial strains in the native phycosphere (mini ecosystem-surrounding microalgal cell walls) may improve the growth of microalgae *C. vulgaris* (e.g. *Flavobacterium, Hyphomonas, Rhizobium, Sphingomonas*). In contrast, others may be inhibitory (*Microbacterium* and *Exophiala*), illustrating that not all interactions need to be mutualistic. It was shown that a co-culture of *Scenedesmus sp*. and *Paenibacillus sp*. was more successful than axenic culture in removing organic contaminants, total dissolved solids (TDS), COD and heavy metals, as well as showing the highest reduction in cytotoxicity and genotoxicity (Kumari et al., 2016). These studies point to interspecies interactions between bacteria and microalgae, which may impact a photobioreactor's performance.

Outcomes of the Smart Use of Resources priority area of application are development of new materials, products, applications and services supporting smart use of resources in the circular economy, development of technological and biological materials, systems for preventing waste generation, systems for quality recycling (upcycling) and re-use and efficient use of resources including water, development of technologies, components and systems for efficient energy use and for the acquisition of alternative fuels, development and production of components and systems for utilising renewable energy sources (energy conversion, distribution and storage).

SMALL CELLULAR PARTICLES AS MEDIATORS OF CELL – CELL COMMUNICATION

There is a critical need for basic science and engineering research that would lead to improvements in scalable technologies for biomanufacturing of delivering particles (Estes et al., 2022) and enrichment of their inner compartment with biogenic molecules (Hermann et al., 2021). Cell - cell communication could be key in opening the "black box" of basic ecological elements and could serve as a lever to equilibrate the state of the living systems. The algae–bacteria interactions are considered promising for biotechnology, as many recent studies have shown a positive effect of algae–bacteria symbiosis on algal growth and flocculation processes, which are the essential steps in algal biotechnology (Fuentes et al. 2016). Symbiotic microalgae–bacteria consortia could be utilized to improve microalgal biomass production and to enrich the biomass with valuable chemical and energy compounds.

Evidence on symbiotic communities of microorganisms points to the role of small (micro- and nano-sized) cellular particles (SCPs) shed by microorganisms and cells in their organization and evolution as a community (Figure 1). It is now acknowledged that cells release various types of SCPs (extracellular vesicles (EVs), antibody complexes, lipoproteins, and other particles) capable of transporting different substances, such as proteins, lipids, sugars and nucleic acids (Gill et al., 2019). SCPs are free to move and interact with close by or distant cells. They carry different cargo and can mediate interaction within the same organism or with different organisms and play an important role in the above environmental issues (Thery et al., 2018). As the interaction is based on the mechanism that is shared between all life domains – i.e. fission and fusion of biological membranes – it offers possibility of controlled intervention and presents the base of our mechanism for climate resilience. EVs have been implicated in many aspects of cell physiology, such as stress response, intercellular competition, lateral gene transfer (via RNA or DNA), pathogenicity and detoxification (Gill et al., 2019). Although microalgal SCPs were first observed in the 1970s (Aaronson, 1971; McLean et al., 1974), and have recently become a subject of further interest (Bozic et al., 2022; Picciotto et al., 2021; Adamo et al., 2021), their roles in communities are not yet fully understood. It is therefore of utmost importance to enhance our understanding of intercellular communication mechanisms, and we expect that this would have implications across multiple fields of science. Intercellular interactions at the microscopic scale connect different life domains and thereby enable application of the same basic principles and similar methods for supporting the microalgal communities as well as processing of natural sources for different uses in maintenance of health (Romolo et al., 2022).

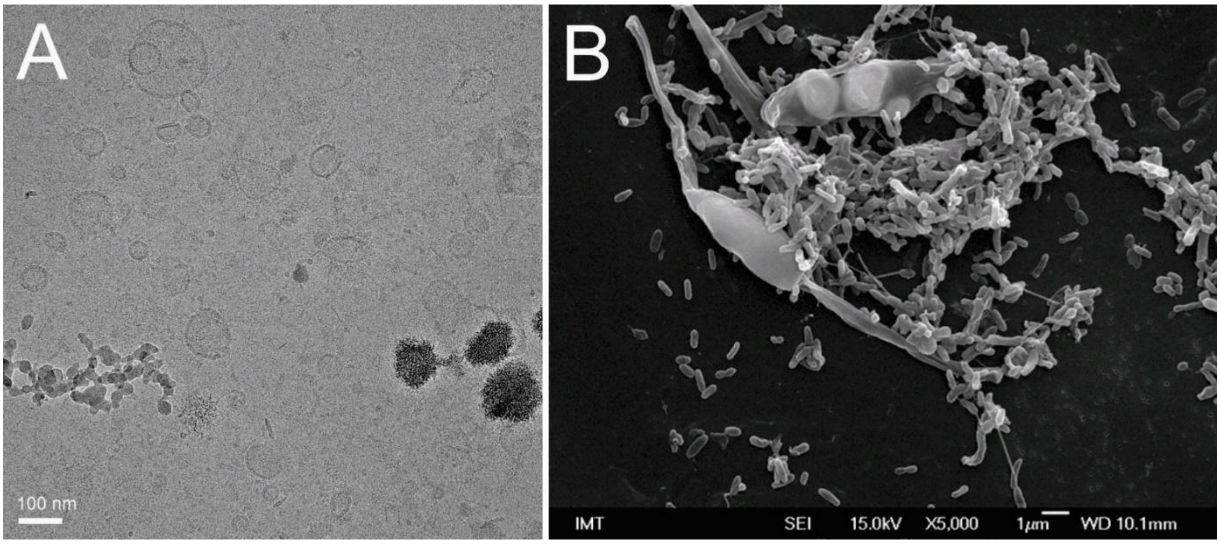

Figure 1. Scanning electron micrograph of isolated SCPs from conditioned media of microalgae *Phaeodactylum tricornutum* (A) and microalgae with numerous bacteriae in the culture (B). A: from (Kralj-Iglič et al., 2022). The sample was prepared for SEM imaging (B) as described in (Božič et al., 2022).

Natural sources are known to be rich with bioactive compounds (*e.g.* lipids, terpenes, polyphenols, flavonoids, etc.), with some of these substances having antibacterial and antioxidant properties which are likely to be captured and transported by SCPs within the body (Jeran et al., 2023). Since SCP field is a relatively new rapidly developing field the key mechanisms are not yet fully understood and the methods for SCP harvesting, and characterization and extraction of the compounds are not yet being full explored. Furthermore, interpretation of the results on new substances in connection with other parameters of the medium urgently need improvements and clarifications. As natural sources contain molecules that proved to have beneficial effects on health, it is indicated that SCP production in these sources should be artificially increased. Formation of hybrid particles by processing of material from natural sources and lipids (hybridosomes) is expected to capture the substances of the natural sources with health-beneficial effects, increase the yield (*e.g.* by bio-stimulants) and render material with better controlled properties. In order to be up-taken by cells, particles should be small (i.e. submicron-sized). In extrusion, the vesicles pass several times (extrusion cycles) through a membrane of defined pore size, to render the size distribution more uniform (Meure et al., 2008; Olson et al., 1979). Nebulization is a particularly interesting route of administration. In the context of lung damage, given its excellent performance in terms of the bioavailability, the drug is delivered to the targeted cells in the lung (Frolich, 2021). Also, intranasal administration of EVs has been established as an effective and reliable way to bypass the blood-brain barrier and deliver drugs to the central neural system (Herman et al., 2021).

Communities of microorganisms managed or functionally enhanced through a greater understanding of SCP communication have a number of extraordinary advantages, key among them being scalability and cost. While central wastewater treatment, and bioremediation often require large-scale investment and equipment, nature-based solutions may be much cheaper and easier to scale, including to the individual household level. From a spatial perspective one can distinguish between macro and micro remediation methods and contexts, and also along the social-ecological, natural and technical axes differentiating resilience management at city and household scales, while all scales are governed by the mechanisms at the cellular level. Extractions with liquids in the subcritical (water) and supercritical (CO_2) state are currently among the most intensively studied technologies of so-called green chemistry. In addition to high ecological (non-toxicity and non-flammability of solvents) and economic (availability of solvents) acceptability, they are most distinguished by new usability potentials, including in the areas of functional foods, cosmetic and pharmaceutical ingredients. Macro and micronutrients as well as the antioxidants present in microalgae could in the future provide food or food additives. Communal wastewater containing significant amount of phosphorus and nitrogen, which are a major cause of eutrophication in water bodies could be bioremediated using microalgae, producing biomass in the process. Microalgae are a reservoir for identifying and extracting biologically active substances like pigments, vitamins, phenolics, squalene, mycosporine-like amino acids, all known as antioxidant agents, as well as phytosterols, with the potential to act as pharmaceuticals, nutritional supplements, and cosmetics.

HYBRIDOSOMES FOR SCALABLE DELIVERY TO CELLS

Efforts are being invested into development of methods for harvesting and characterization of nano-sized particles, however, a golden standard method has not yet been acknowledged. Suspensions of liposomes are dynamic systems subjected to self-assembly according to the minimization of the free energy. They are not particles with fixed identity and are prone to transformations of shape, size, and composition during the processing of samples. By appropriate selection of the cells from which SCPs derive, various conjugating targeting moieties have been developed (Hermann et al., 2021) *e.g.* SCPs from natural sources, artificial vesicles produced from lipids (liposomes) and their combinations (Piffoux et al., 2018). Hybridosomes mimic natural ones in terms of biological activity but can be synthesized in higher amounts and filled intentionally with specific and desirable cargo (Maurer et al., 2001, Belliveau et al., 2012). Combination of vesicles of natural origin and synthetic ones would circumvent robust cellular manipulation needed for isolation of natural extracellular particles (Belliveau et al., 2012, Marcus et al., 2013) (which is usually expensive and time consuming) and enable artificial nanocarriers to entrap biologically active molecules (Mashima and Takada, 2022). Employment of this nanotechnological strategy could generate efficient and reproducible biocompatible carriers for downstream applications. The idea is to enclose the natural compounds into vesicles composed mainly of artificially added lipids.

CONCLUSIONS

Microalgae are amazing creatures, which engineer their composition to adapt to the surroundings. Some algae can swim hundreds of their lengths in a second for the ability of their flagella. They can drop the flagella and build them again in hours. They have a high ability to duplicate their number. Their communities can dwell in symbiosis with the urban environment, where they can provide valuable presence of living beings and aesthetic pleasure for their colours. However, the algal communities and their communication with themselves and other microorganisms remain the most important element to benefit from their capacity to cope with a variety of environmental stress factors while producing bioactive substances.

ACKNOWLEDGEMENTS

Authors acknowledge support of Slovenian Research Agency, grants P3-0388, J3-3066, J2-4447 and J2-4427.

REFERENCES

1. *Aaronson S, The synthesis of extracellular macromolecules and membranes by a population of the phytoflagellate Ochromonas Danica. Limnol Oceanogr, 1971,16:1–9. https://doi.org/10.4319/lo.1971.16.1.0001*
2. *Adamo G, Fierli D, Romancino DP, Picciotto S, et al. Nanoalgosomes: Introducing extracellular vesicles produced by microalgae. J Extracell Vesicles, 2021; 10:e12081. https://doi.org/10.1002/jev2.12081*
3. *Armingol E, Officer A, Harismendy O, Lewis NE, Deciphering cell–cell interactions and communication from gene expression. Nat Rev Genet, 2021,22:71–88. https://doi.org/10.1038/s41576-020-00292-x*
4. *Atanasova N, Castellar JAC, Pineda-Martos R, et al., Nature-Based Solutions and Circularity in Cities, Circ Econ Sustain 1, 2021: 319–32, https://doi.org/10.1007/s43615-021-00024-1*
5. *Belliveau NM, Huft J, Lin PJ, et al., Microfluidic Synthesis of Highly Potent Limit-size Lipid Nanoparticles for In Vivo Delivery of siRNA. Mol Ther Nucleic Acids, 2012,1(8): e37. https://doi.org/10.1038/mtna.2012.28*
6. *Božič D, Hočevar M, Jeran, M, et al., Scanning electron microscopy of microorganisms growing in co-cultures with microalgae. Proc Socrat Lect 2021, 6:206. DOI: 10.55295/PSL.2021.D*
7. *Božič D, Hočevar M, Jeran M et al., Ultrastructure and stability of cellular nanoparticles isolated from Phaeodactylum tricornutum and Dunaliella tertiolecta conditioned media. Open Res Europe. 2022; 2, 121. https://open-research-europe.ec.europa.eu/articles/2-121/v1*
8. *Brennan L, Owende P, Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev, 2010, 14:557–577. https://doi.org/10.1016/j.rser.2009.10.009*
9. *Cavicchioli R, Ripple WJ, Timmis KN, et al. Scientists' warning to humanity: microorganisms and climate change. Nature reviews. Microbiology, 2019, 17(9), 569–586. https://doi.org/10.1038/s41579-019-0222-5*
10. *Combarnous Y, Nguyen TMD. Cell Communications among microorganisms, plants, and animals: Origin, evolution, and interplays. Int J Mol Sci, 2020; 21: 8052. https://doi.org/10.3390/ijms21218052*
11. *Craggs R, Sutherland D, Campbell H. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J Appl Phycol, 2012, 24:329–337. https://doi.org/10.1007/s10811-012-9810-8*
12. *Estes S, Konstantinov K, Young JD, Manufactured extracellular vesicles as human therapeutics: challenges, advances, and opportunities, Curr Opin Biotechnol, 1011, 77 :102776, https://doi.org/10.1016/j.copbio.2022.102776*
13. *Field CB, Behrenfeld MJ, Randerson JT, Falkowski P, Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science 1998, 281(5374):237-40. https://www.science.org/doi/10.1126/science.281.5374.237*
14. *Frolich E, Therapeutic Potential of Mesenchymal Stem Cells and Their Products in Lung Diseases-Intravenous Administration versus Inhalation. Pharmaceutics, 2021, 13:232. https://doi.org/10.3390/pharmaceutics13020232*
15. *Fuentes JL, Garbayo I, Cuaresma M, et al., Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and Associated Compounds. Marine Drugs, 2016, 14(5), 100. https://doi.org/10.3390/md14050100*
16. *Geens T, Goeyens L, Covaci A., Are potential sources for human exposure to bisphenol-A overlooked? Int J Hygiene Environ Health, 2011, 214(5), 339–347. https://doi.org/10.1016/j.ijheh.2011.04.005*
17. *Gill S, Catchpole R, Forterre P, Extracellular membrane vesicles in the three domains of life and beyond. FEMS Microbiol Rev,2019, 43:273–303. https://doi.org/10.1093/femsre/fuy042*
18. *Herman S, Fishel I, Offen D. Intranasal delivery of mesenchymal stem cells-derived extracellular vesicles for the treatment of neurological diseases. Stem Cells, 2021, 39: 1589–1600.*

https://doi.org/10.1002/stem.3456
19. Jeran M, Romolo A, Spasovski V, et al., Small Cellular Particles from European Spruce Needle Homogenate. Int J Mol Sci, 2023, 24(5):4349. https://doi.org/10.3390/ijms24054349
20. Ji X, Jiang M, Zhang J, et al., The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. Bioresour Technol, 2018, 247 : 44–50. https://doi.org/10.1016/j.biortech.2017.09.074
21. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and analysis of cell-cell communication using CellChat. Nat Commun, 2021, 12 :1088. https://www.nature.com/articles/s41467-021-21246-9
22. Kralj-Iglič V, Bedina Zavec A, Božič D, et al., Scanning electron microscope images of Dunaliela tertiolecta and Phaeodactylum tricornutum cultures and scanning electron microscope images and cryogenic electron microscope images of isolated small cellular particles from respective conditioned media. Zenodo. https://doi.org/10.5281/zenodo.6908895
23. Kumari M, Pooja G, Indu ST, Landfill leachate treatment using bacto-algal co-culture: an integrated approach using chemical analyses and toxicological assessment. Ecotoxicol Environ Safety, 2016, 128: 44-51. https://doi.org/10.1016/j.ecoenv.2016.02.009
24. Langergraber G, Castellar JAC, Andersen TR, et al., Towards a cross-sectoral view of nature-based solutions for enabling circular cities. Water 2021, 13: 2352. https://doi.org/10.3390/w13172352
25. Liu R, Li S, Tu Y, Hao X, Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. J Environ Man, 2021, 285 :112149. https://doi.org/10.1016/j.jenvman.2021.112149
26. Marcus ME, Leonard JN, FedExosomes: Engineering Therapeutic Biological Nanoparticles that Truly Deliver. Pharmaceuticals, 2013; 6(5):659-680. https://doi.org/10.3390/ph6050659
27. Maryjoseph S, Ketheesan B, Microalgae based wastewater treatment for the removal of emerging contaminants: A review of challenges and opportunities. Case Stud Chem Environ Eng, 2020, 2:100046. https://doi.org/10.1016/j.cscee.2020.100046
28. Mashima R, Takada S, Lipid Nanoparticles: A Novel Gene Delivery Technique for Clinical Application. Curr Issues Mol Biol, 2022, 44(10):5013-5027. https://doi.org/10.3390/cimb44100341
29. Maurer N, Wong KF, Stark H, et al., Spontaneous entrapment of polynucleotides upon electrostatic interaction with ethanol-destabilized cationic liposomes. Biophys Journal, 2001, 80(5), 2310–2326. https://doi.org/10.1016/S0006-3495(01)76202-9
30. Meure LA, Foster NR, Dehghani F, Conventional and dense gas techniques for the production of liposomes: a review. AAPS Pharm Sci Tech, 2008; 9: 798-809. https://doi.org/10.1208/s12249-008-9097-x
31. Muñoz I, López-Doval JC, Ricart M, et al., Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (northeast Spain). Environ Toxicol Chem, 2009, 28 : 2706.https://doi.org/10.1897/08-486.1
32. Noguera-Oviedo K, Aga DS, Lessons learned from more than two decades of research on emerging contaminants in the environment. J Hazard Mater, 2016, 316 :242–251. https://doi.org/10.1016/j.jhazmat.2016.04.058
33. Norvill ZN, Shilton A, Guieysse B. Emerging contaminant degradation and removal in algal wastewater treatment ponds: Identifying the research gaps. J Hazard Mat, 2016, 313, 291–309. https://doi.org/10.1016/j.jhazmat.2016.03.085
34. Olson F, Hunt CA, Szoka FC, et al., Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. BBA, 1979, 557: 9–23. https://doi.org/10.1016/0005-2736(79)90085-3
35. Park JBK, Craggs RJ, Shilton AN, Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol, 2011, 102:35–42. https://doi.org/10.1016/j.biortech.2010.06.158
36. Picciotto S, Barone ME, Fierli D, et al., Isolation of extracellular vesicles from microalgae: towards the production of sustainable and natural nanocarriers of bioactive compounds. Biomater Sci, 2021, 9:2917–2930. https://doi.org/10.1039/D0BM01696A

37. *Piffoux M, Silva AKA, Wilhelm C, et al., Modification of Extracellular Vesicles by Fusion with Liposomes for the Design of Personalized Biogenic Drug Delivery Systems. ACS Nano, 2018, 12:6830–6842. https://doi.org/10.1021/acsnano.8b02053*
38. *Prosenc F, Piechocka J, Škufca D, et al., Microalgae-based removal of contaminants of emerging concern: Mechanisms in Chlorella vulgaris and mixed algal-bacterial cultures, J Hazard Mat, 2021, 418 :126284, https://doi.org/10.1016/j.jhazmat.2021.126284*
39. *Rahman MA, Aziz MA, Al-khulaidi RA, et al., Biodiesel production from microalgae Spirulina maxima by two step process: Optimization of process variable. J Radiat Res Appl Sci, 2017, 10:140–147. https://doi.org/10.1016/j.jrras.2017.02.004*
40. *Ramanan R, Kim BH, Cho DH, et al., Algae-bacteria interactions: Evolution, ecology and emerging applications. Biotechnol Adv. 2016, 34(1):14-29. https://doi.org/10.1016/j.biotechadv.2015.12.003*
41. *Romolo A, Jan Z, Zavec Bedina P, et al., Assessment of Small Cellular Particles from Four Different Natural Sources and Liposomes by Interferometric Light Microscopy. Int J Mol Sci. 2022; 23: 15801. https://doi.org/10.3390/ijms232415801*
42. *Sarmento H, Morana C, Gasol J, Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality. ISME J, 2016, 10:2582–2592. https://doi.org/10.1038/ismej.2016.66*
43. *Sutherland DL, Ralph PJ, Microalgal bioremediation of emerging contaminants - Opportunities and challenges. Water Res, 2019, 164:114921. https://doi.org/10.1016/j.watres.2019.114921*
44. *Sutherland DL, Park J, Heubeck S, et al., Size matters—Microalgae production and nutrient removal in wastewater treatment high rate algal ponds of three different sizes. Algal Res 2020, 45 : 101734. https://doi.org/10.1016/j.algal.2019.101734*
45. *Škufca D, Prosenc F, Kovačič A, et al., Removal of contaminants of emerging concern in algal photobioreactors: From lab-scale to pilot-scale. Proc Socrat Lect 2021, 6 :207. DOI: 10.55295/PSL.2021.D*
46. *Théry C, Witwer KW, Aikawa E, et al., Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guideline. J Extracell Ves. 2018; 7: 1535750. https://doi.org/10.1080/20013078.2018.1535750*
47. *Tolboom SN, Carrillo-Nieves D, de Jesús Rostro-Alanis M, et al., Algal-based removal strategies for hazardous contaminants from the environment—A review. Sci Total Environ 2019, 665 :358–366. https://doi.org/10.1016/j.scitotenv.2019.02.129*
48. *Vehar A, Kovačič A, Hvala N, et al., Fate of bisphenols during conventional wastewater treatment. Proc Socrat Lectures 2021, 6 :57–62. https://doi.org/10.55295/PSL.2021.D.008*
49. *Yadav D, Rangabhashiyam S, Verma P, et al., Environmental and health impacts of contaminants of emerging concerns: Recent treatment challenges and approaches. Chemosphere 2021, 272: 129492. https://doi.org/10.1016/j.chemosphere.2020.129492*
50. *Zimmerman N, Izard J, Klatt C, et al., The unseen world: environmental microbial sequencing and identification methods for ecologists. Front Ecol Environ, 2014, 12, 224–231. DOI: 10.1890/130055*