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Strategic Designing of The Algal Culture System to Obtain Valuable Products

KEYWORDS: algae, biodiesel, protein, nano-particles

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ABSTRACT *Algae are a renewable resource of many products. This review examines the role of algae as a source of bio-diesel and proteins. The extraction of lipids is proposed to be coupled with biological synthesis of metallic nano-particles and subsequent extraction of proteins from the remaining bio-mass. The low cost engineering aspects are discussed and dealt with in brief.*

INTRODUCTION:

Algae have a unique inbuilt biochemical machinery fuelled by sunlight, water and gaseous carbon dioxide to convert the carbon source into biomass (44), quite similar to the process that occurs in aquatic or terrestrial plants which are however stringent upon certain other nutrient requirements like nitrogenous and phosphorus compounds supplied to them as fertilizers/manures. More over algae reproduce at a much higher rate bringing about a net increase in the turn over rate of biomass (2). In many parts of the world, this algal bio reactor operates spontaneously in ponds and lake systems spread as either freshwaters or else marine waters (48). Algae cultivation on the commercial scale is currently being carried out in much lesser labor intensive platforms called as Photo bioreactors, which are preferred due to lesser risks of contamination and larger productivity due to ability to operate at various control parameters (2,4,48,47). Chisti et al.(2007) have thoroughly examined the biofuel producing ability of microalgae and have concluded that biodiesel is unarguably the most expensive product that can be harnessed from the algal cultivation system, besides which various other kinds of biofuels like bioethanol, biobutanol, bio hydrogen etc. can also be obtained. The residual biomass serve as biofertilizers, cattle feed, fish feed etc. The food grade algae are converted to nutraceuticals like protein supplements and anti-oxidants like the carotenoids "Lutein" or "Astaxanthin", omega-3-fatty acids, vitamins etc. aimed for human consumption (48). This review discusses how the algal system can be exploited for many more products by manipulating engineering aspects at the nutrient level to generate novel products like nanocomposites or the genetic engineering operating at the molecular level to obtain therapeutic proteins or vaccines.

1. BIODIESEL FROM ALGAE

Algae incorporate a huge amount of lipids and oils in their biomass. (1, 2, 3).The twenty-first century sees algae derived fuels much superior to that of other in-organic sources like petroleum fuels (3, 4) or biological sources like plant-derived fuels (2, 5, 6). Algae are photo synthetically more efficient as compared to land-plants (7). More so contradictory to the general notion, harvesting algae can be a very cheap process that can use industrial effluents as source of nutrients and carbon-dioxide (8, 9). However the major constraint in scaling up of the bio-diesel production technology especially those using industrial effluents and open pond system is microbial/bacterial contamination (10). So, it becomes inevitable to design methods that enhance the biodiesel production as well as take care of the microbial pollutants.

2. ALGAE AS SOURCE OF NANO-PARTICLES:

Intracellular synthesis of nanoparticles has been observed to be mediated by algae in many cases (17) and a few also exhibit extra-cellular synthesis (11). Extracellular synthesis has many merits including abatement of toxicity towards the algal cells. Luangpipat et al., have reported the intracellular production of gold, platinum, palladium, ruthenium, rhodium and iridium in Chlorella vulgaris (20). Varied structures of nano-materials like nano-rods (21) and nano-prisms (22) have also been reportedly synthesized using biological synthesis method and algal extracts.

Silver nanoparticles are being very extensively used in various products and therefore need to be synthesized through ecologically benign techniques. They show very high thermal and electrical conductivity which is an important reason for their application in biomedical field in very complex technologies such as enzyme electrode or bio sensing of physiologically relevant molecules (12, 45). The antibacterial, antifungal as well as anti-viral properties have been well established (28,29,45). The AgNPs exhibit strong antibacterial effect on the enteric bacteria which predominate sewage waters and industrial waste streams (28). A rather distinguishing feature of the AgNPs is that they exert selective toxicity against a toxin secreting, harmful algae "Microcystis aeruginosa" which very often contaminate the algal culture systems (50).

Many strains of marine algae and also fresh water algae e.g. Chlorococcum humicola have shown to have potential for silver nano-particle synthesis (11). Barwal et al., also have reported the intracellular and extra cellular AgNP production in the microalga Chlamydomonas reinhardtii. The algal species mostly reported to synthesize nano-particles belong to the chlorophycea e.g. Chlorella spp. (39) and to other phyla like Shewanella spp. (40).

Some studies have however indicated a sharp decline in the cellular viability of algae in the presence of extracellular NPs(49) but it is now known that various mechanisms to mitigate this is present in the algal physiology itself (27). Further to overcome this by engineering the bioreactor system, it might be strategic to remove and extract the synthesized nano particles at short intervals and produce an alternating batch mode and continuous culture system.

3. CAN WE COMBINE BIODIESEL PRODUCTION WITH NANO-PARTICLE SYNTHESIS?

Biological synthesis offers a much more economical mode of nano-particle synthesis, reducing the investment by manifolds as compared to other physico-chemical means (23). A combinatorial methodology can have a profound impact on the industrial set ups for biodiesel production for which the rationale is also quite supportive. Heavy metals are known to act negatively on the nitrate reductase activity of algae (24). The effect of metallic nano-particles is yet to be elucidated but in the current light, it is quite evident that addition of inert metallic nano-particles into the algae culture might as well create a condition of nitrate starvation around the algae and this might as well enhance the oil content in the algal

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biomass (25). Instead of a pure photosynthetic approach, a photosynthetic-fermentative approach (26) can be more productive while combining the metal nano-particles with the biodiesel production. The probability is also high that nanoparticle synthesis coupled with a bioreactor to grow algae for biodiesel production might also provide tolerance to waste water rich in ammonia and other nitrogenous compounds. Studies have shown, nano-toxicity is reduced many folds by the synthesis of organic compounds (27) mostly acids by the algae. Further more if we consider a photosynthesis-fermentative approach rather than purely photosynthetic one, it is apprehended that the formation of reactive oxygen species by Ag NPs is reduced by many fold as the surrounding oxygen concentrations are reduced.

One more mechanism of stable nanoparticle synthesis from the algal fermentation tanks could be enzymatic action. Under fermentative conditions and nitrogen limitation conditions, the activity of L-asparaginase enzyme in algae like Chlamydomonas sps. is reportedly enhanced many folds [51] .Thus, increasing the amounts of aspartic acids/acidic amino acids can help reduce the silver salts to form zero-valent silver nanoparticles. Further a good economic approach would be to extract this enzyme (as the enzyme content increased) from the alga; asparaginase being an anti-tumor substance and FDA approved drug. Yet another algal enzyme that is known to create reducing conditions is –urease. [52].This enzyme presents a potential mechanism of nano-particle synthesis in a bioreactor system as most bioreactors are fed urea as nitrogen source.

Another aspect which is quite favorable for the approach is that by merging both the bio processes is that it can help maintain the culture for a longer time. The antimicrobial attributes of the silver nano-particles can exert a negative impact on the growth of bacteria and protozoa that contaminate the algal culture system especially while using industrial effluents as media or nutrient source (28,29,30). The extraction technology of either of the process does not interfere with one another. More so, the proposed technique has a rather simpler method of extraction of the produced nanoparticles than using the conventional bacteria or fungi for biosynthesis. The algal cells can be ruptured easily (in the case of intracellular synthesis) else the nanoparticles agglomerate easily (in the case of extracellular synthesis). As the water used thus, can be again set for reuse in photosynthesis, there seems to be no economical constraints in the approach.

Various studies have been carried out to genetically modify algae for obtaining useful products from algae, especially fermentation products like ethanol with biodiesel production e.g. by the chloroplast engineering that couples photosynthesis by algae to a bacterial gene for ethanol production (31).

4. PRODUCTION OF PROTEINS FROM ALGAE:

The major challenges faced by industry while producing proteins of therapeutic or economical value are high capital and media costs. The proteins produced by mammalian cell culture are very expensive due to the complexity of mammalian cell culture. The bacteria on the other hand are often inefficient at producing properly folded complex proteins, requiring denaturation and renaturation steps that add significant costs to the process of protein production. Algae being eukaryotic and having similar protein folding mechanisms as the human, invariably is our best option as already been pointed out by Stephen Mayfield, professor of biology at university of California,San diego that seven diverse human therapeutic proteins could be produced in Chlamydomonas reinhardtii and with levels sufficient for commercial production (41). It is economical as compared to other sources of protein production such as transgenic mammalian cells or E.coli (bacteria). Also algal-produced proteins in their study showed biological activity comparable to the same proteins produced by traditional commercial techniques. And because algae cells can be grown cheaply and quickly, doubling in number every 12 hours, they noted that algae could be superior to current biological systems for the production of many human therapeutic proteins. The percentage of human proteins produced in their algal cultures that were properly folded in three dimensions was comparable to the fraction produced by mammalian cell cultures and much better than that produced by bacterial systems. And because algae generate their energy from sunlight and have relatively simple nutrient needs, the costs for using them at large scale to commercially produce human proteins should be much lower than for mammalian cell culture, which required expensive fermentation facilities. Seven different theraputic proteins naming human interferon β1 for treatment of Multiple Sclerosis, human erythropoietin or EPO for increase of red blood production in patients undergoing chemotherapy , human proinsulin for Type 1 diabetes , human vascular endothelial growth factor or VEGF , domains 10 and 14 of human fibronectin were produced from in Chlamydomonas reinhardtii .

The principle behind the recombinant protein production is that of chloroplast engineering. This is because the nuclear genes are often subject to gene silencing upon exogenous gene insertion. Algae under nutrient stress conditions do not only accumulate more lipids but few important proteins also like the heat shock proteins. These are amongst few algal proteins that can be harnessed after lipid extraction.

Mayfield et al. have already demonstrated the production of human antibodies and serum amyloid protein, which are known to be very complex proteins from the algal system.

5. FUTURE DIRECTIONS AND CHALLENGES

Algal systems are very inexpensive bioreactors. They can be used for bio fuel production as well as therapeutic proteins and nanoparticles production and in order to harness both the later products from it, a single bioreactor system may utilize the bio fuel producing strains. The nano-particle synthesis is best when produced extra cellular so that it is extractable by physical separations. Further we should assess the rate of formation of the nano-particles which should not exceed the ecotoxicity levels against algal growth. For instance Hazani et al. (2013) have reported that greater about 100 mg/L silver nano particles is known to interfere with the cell-viability of most green alga. Discussing the second aspect which is the production of proteins of therapeutic value, it is highly recommended that we aim at heat-stability of the therapeutic proteins such that the proteins can with stand high temperature conditions of the commonly used processes for lipid extraction or even the transesterification process without getting denatured. Although alga-based proteins and vaccines are generally heat stable but it could be made economical if we are able to harness it after the removal of lipids. Such multifunctional proteins are to be tested comprehensively in the in vivo models prior to be used as therapeutics. Chloroplast engineering has many problems and it should be dealt with for instance we generally compromise with the photosynthetic ability in the process and the biomass generation rate has to be maintained constant which might necessitate incorporation of simple sugars into the nutrient medium. The perplexing scenario is that sugars here are necessary for protein production rather than other fermentative products. Thus, there is a long road ahead to realize the full potential of an algal system.

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