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Factors to Consider in Algal-Strain Selection for Commercial Manufacture of Biofuels and Co-Products

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Introduction

Commercialization of algal-based processes for biofuels and co-products is constrained upstream during cultivation by low productivity, contamination by invasive species, unsustainable and inefficient supply of nutrients (e.g. nitrogen and phosphorous), inorganic carbon, and water, exposure to environmental factors, and limited available land acreage for inoculum and primary cultures. It is also constrained downstream by costly and inefficient harvesting, product extraction, thermochemical conversion, hydrotreatment, and upgrading. Overcoming these obstacles to commercialization requires not just piecemeal incremental improvements, but rather a comprehensive and fundamental re-consideration regarding the selected algae and its associated cultivation, harvesting, biomass conversion, and refinement. Factors pertaining to algal-strain selection for commercial operations are presented below.

Selection of Candidate Algae

For the manufacture of lipid-based precursors to biofuels, many of the algal industry, research community, and state and federal funding agencies like the U.S. Department of Energy (DOE) have emphasized and funded experimental research and development studies involving the large-scale, outdoor cultivation of select, oleaginous, eukaryotic, and well-characterized microalgal strains (i.e. *Scenedesmus sp., Chlorella sp.*) whose unicellular nature, small dimensions, preference for freshwater and neutraphillic growth conditions, and relatively spheroidal morphology puts them at an inherent disadvantage. Marine strains like *Nannochloropsis sp.* and salt-adapted variants of the aforementioned strains are exceptions to this.

Small Dimensions and Spherical Morphology vs. Large Dimensions and Filamentous Morphology

Compared to large and filamentous algal morphology, small dimensions and spherical algal morphology are advantageous for the following reasons:

a) They increase bubble-algal adhesion rates during dissolved air-flotation-based harvesting methods.

b) They are more amenable to novel ultrasonic acoustic wave focusing-based harvesting methods and to TAG-quantification via rapid, non-destructive analytical methods like H-NMR which rely on small, spherical algae bending the respective electro-magnetic fields.

c) They may enhance algal light transfer and mass-transfer of dissolved nutrients and waste products across the cellular wall and membrane.

d) Their morphology does not directly contribute to increases in hydrodynamic viscosity which hinders momentum, mass, and heat transfer. For instance, filamentous morphology, much like the hyphae of late-stage fungi, promote algal entanglement of the blades and rotors of circulating paddle-wheels used to agitate raceway ponds. However, small dimensions and spherical algal morphology are also disadvantageous for the following reasons:

i. They make the algae more susceptible to invasion and contamination by undesirable species (i.e. fungi, rotifers, bacteria, undesirable phototrophs) who prey on the microalgae, release toxins or other growth inhibitors, and/or compete for light and nutrients. As a result, commercialization is burdened by the added costs associated with contamination detection and monitoring (i.e 16s and 18s rDNA isolation and PCR amplification for genotyping, fiber optic sensors for remote measurement of reflectivity to detect fluorescence of chlorophyll-metabolic by-products arising from predation) and intermittent operations due to shut-downs of large, raceway open-ponds that have “crashed”.

ii. They generally lead to harvesting inefficiencies which manifest as reduced settling rates following flocculation, dissolved air-flotation, acoustic wave focusing, centrifugation, etc. and as increased reversible cake and irreversible pore fouling rates following membrane ultra filtration. As a result, commercialization is burdened by the added operating cost of energy consumption and direct labor, as well as capital costs of acquisition, freight, installation, and validation associated with harvesting equipment. This in turn limits the efficiency of recycling water and nutrients after harvesting.

Eukaryotic vs. Prokaryotic

Compared to wild-type prokaryotic cyanobacteria (a.k.a blue-green algae), many of the aforementioned conventional eukaryotic algal strains offer the following advantages

a) With the exception of photosynthetic pigments like phycobilisomes (i.e. phycocyanin, phycoerythrin, etc.) that cyanobacteria and red-algae like *Gualdieria sp*. make, the eukaryotic algae presently exhibit higher native productivity kinetics for both high-value products (i.e. omega-3 free fatty acids, lutein, astaxanthin) and lipid-precursors to low heating-value commodity biofuels (i.e. TAG, DAG, MAG) with low heating values.

b) Unlike some filamentous cyanobacteria like wild-type *Anabaena sp.*, they are not known to produce toxins. These render the harvested biomass unfit for animal consumption and, like the aforementioned secreted polysaccharides in saltwater, also represent a metabolic diversion of carbon and energy away from desired products.

However, compared to prokaryotic algae, eukaryotic algae offer the following disadvantages:

a) They possess genomes and transcriptomes of greater complexity in terms of regulation and the presence of intron-sequences,

b) They possess cell walls of greater complexity in terms of structure and composition,

c) They possess organelles (i.e. nucleus, mitochondria, chloroplast) of great complexity in terms of offering more genomic targets which simultaneously offer advantages of appropriate post-translational folding and glycosylation of heterologously expressed high-value, therapeutic and diagnostic antibody proteins and disadvantages of low copy-number and yet-to-be elucidated and controlled gene silencing mechanisms.

d) They generally feature lower growth productivity rates and doubling times. These distinctions therefore make eukaryotic algae, even those with filamentous morphology, less amenable to genetic transformation, stable heterologous integration and expression of recombinant DNA, and secretion of desired products into the extra-cellular space. These further limit their use in the immediate future for the manufacture of secreted, pure, volatile, high heating-value hydrocarbons. Such products, which conceptually are more easily manufactured by simpler cyanobacteria, are more cost-effective because they require absolutely no downstream hydrothermal liquefaction, upgrading, and refining processes typically reserved for conversion of the highly acidic, complex, unstable, O2-rich bio-oil derived from eukaryotic algae.

Marine vs. Freshwater

Compared to cultivation of marine algae, cultivation of freshwater algae like *Scenedesmus sp*. And Chlorella sp. is advantageous for the following reasons:

a) The larger-diameter "cap" air-gas bubbles delivered via orifice-spargers deform and coalesce more in freshwater and either remain or become large enough to supply sufficient momentum from buoyancy forces for agitation and O2-stripping.

b) Gases like CO^2 are generally more soluble in freshwater than they are in saltwater, freshwater, unlike saltwater, does not promote desiccation which causes some algal strains to increase their secretion of extra-cellular polysaccharides. These secretions may facilitate flocculation-mediated harvesting by bridging electrostatic charge forces. Conversely, however, these increase hydrodynamic liquid viscosity and lower gas-liquid mass transfer coefficients, hinder membrane-mediates harvesting, increase long-term risk of contaminants that use secretions as organic carbon source, and represent an undesirable metabolic diversion of carbon and energy away from desired products.

d) Freshwater is devoid of salts impurities, and other surfactants that lowers the gas-liquid mass transfer coefficient (Kl).

e) Freshwater does not require the purchase of expensive artificial salts when cultivating marine strains in facilities that are not co-located next to salt lakes or oceans. However, compared to cultivation of marine algae, cultivation on freshwater algae is disadvantageous for the following reasons:

i. CO^2-gas bubbles delivered via micro-diffusers and membranes for delivery of inorganic carbon and pH control coalesce more in freshwater and will suffer from high rise velocity, low gas-holdup, low surface tension, and low specific surface area (and, consequently, lower overall gas-liquid mass transfer coefficient KLa,

ii. Cultivation using freshwater diverts water away from human consumption or other competing applications.

iii. The coalescent, relatively large-sized bubble flow regime in freshwater reduces efficiency associated with coagulation-, flocculation-, and dissolved air flotation-based harvesting processes. freshwater with low ionic strength allows for more ammoniacal-N to volatilize into the atmosphere, representing an economic loss,

iv. Freshwater with low ionic strength does not offer salt-stress to limit the onset of contamination by non-halotolerant species. Therefore, cultivation of marine or halotolerant algae in coastal regions adjacent to salt lakes and oceans using paddle-wheels, as opposed to sparged air, for agitation of raceway ponds could be more advantageous than cultivation of freshwater microalgae. In conclusion, cultivation of small, spheroidal, freshwater microalgae is both unnecessary and problematic. Commercialization of algal-based products is more likely if focus is re-directed towards cultivation of halotolerant, filamentous, cyanobacteria and microalgae.