INTRODUCTION:

*Dunaliella salina* grows in highly concentrated salt water and is the richest source of natural orange, yellow or red pigmented carotenoids. The antioxidant and pro-retinoic acid activity of carotenoids may protect humans from compromised immune response, premature ageing, cancers, cardiovascular disease and arthritis. The D-Factor is a biorefinery which produces nutraceuticals, chemicals, feed and fuels from *Dunaliella*. 

**Processes that make up the D-Factory.**

- Culture in raceways, with or without PBR inoculation.
- Harvest water-insoluble biomass and dry to a powder.
- Extract powder with scCO₂ or sequentially with solvents to give carotenoid extracts enriched in lipophilic carotenoids, chlorophyll, lipids and defatted powder.
- Process extracts with polar solvents and HPCCC for polar lipids, lutein, zeaxanthin and chlorophylls.
- Process non-polar extracts with solvents and HPLC for all-trans-f-carotene, 9-cis-f-carotene, f-carotene and non-polar lipids.

**Two cultivation settings provide the opportunity for scoping requirements for sustainability**

**NBT, Eilat Israel**

Lined raceways fitted with paddle wheels hold sea- and salt-water. Salt is maintained at 10-15% salinity. Liquid pressurised CO₂ is bubbled in for carbonation for algal growth. Inoculation and scale-up are with smaller raceways. Algae are harvested by partially or completely draining the raceways. Raceways are operated for 300 days per year, in winter (5°C - 15°C) and summer (>40°C). Wastewater contain high salt loads and is treated in aeration and sedimentation ponds then filtered before reuse or discharge to the sea. **Typical Strains:** D. bardawil, DF15, DF17.

**MONZON BIOTECH, Spain**

As above, but raceways have raised sides and a heating system for temperature control in winter, established with excess low grade heat from a 14MW CHP plant, which also supplies flue gas CO₂ from natural gas combustion (up to 7% CO₂). Brine is sourced from shallow underground salt deposits infused with freshwater. Blue water and brine are treated and recombined and fed into the pond. **Typical Strain:** DF40.

**Innovations introduced**

1. **Inoculation with GWP and PBRs**

   - GWP and PBR from A47 erected at Monzon, Spain, for cultivation of *Dunaliella salina* for inoculation trials.

2. **Cell harvesting**

   Cells harvested intact with Evodos technology and membrane pre-concentration allows subsequent controlled cell rupture.

3. **Culture medium recycle**

**FINDINGS:**

- **Strains in a given location** do not influence biomass composition as much as environmental conditions (salt concentration, temperature, light intensity).
- **Algae cultivation— similar to traditional agriculture**— requires substantial energy and material inputs.
  - **Energy:** On-site solar power or renewable steam production from geothermal energy at certain locations can significantly decrease fossil-based energy input and related GHG emissions - up to almost zero.
  - **Freshwater:** Fresh water (or seawater) use may be reduced with efficient medium recycling, reducing costs and environmental burdens. **Land-use:** Wastewater treatment requires land which may be minimised by harvesting cells intact. Raceways require complete ground sealing and could be sited on sealed, disused, industrial brownfield sites instead of agricultural land.
  - **CO₂:** CO₂ from the combustion of natural gas direct flue gas injection does not need flue-gas treatment and does not impact on FDA or regulatory approval for algae-derived products. However if the decarbonisation policy direction initiated today is successfully implemented in the coming decades, increasingly few CO₂ sources will be available for CO₂ utilisation from exhaust gases in the future.
  - **Salt:** Integration with existing facilities for producing salt products or seawater desalination facilities minimises the environmental burdens of energy and material use, impacts on local freshwater availability and disposal of saltwater as well as efforts for regulatory compliance.
  - **Evodos spiral-plate harvesting technology** will harvest cells intact, to recover up to 50% more organic matter from cells. More algal product can be recovered and costs of effluent treatment and medium recycle can be reduced. But the technology is slow (1,000 - 3,000 L h⁻¹) compared to disc-stack centrifuges (12,000 L h⁻¹) and under high ambient temperatures, reduces the concentration of susceptible carotenoid isomers.
  - **Membrane pre-concentration technology** is potentially a low-cost pre-concentration step for harvesting cells before centrifugation, and could permit efficient medium recycling once developed for carotenogenic cells.
  - **Closed PBR cultivation systems** require more energy for algae biomass production than raceways. Since inoculum production comprises only a small fraction of the total algae cultivation, the influence on environmental impacts of algae-based products is small.


**D-FACTORY PARTNERS**


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