

# Nanobubble-Enhanced Algaculture: Nano-aeration and -Carbonation Strategies, with Micro- and Macro-Algae Cultivation and Oxidative Bioremediation Pathways in Soil

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## Abstract

Nanobubble-enhanced algaculture offers a promising pathway to simultaneously increase algal-biomass productivity and expand environmental remediation applications. This study summarises prototype trials of electric-field-driven nanobubble (NB) generation with particular emphasis on nano-carbonation, with applications to micro- and macro-algal cultivation systems and subsequently to soil regeneration. In raceway-pond and retrofitted photobioreactor configurations, nano-aeration and nano-carbonation substantially improved gas-liquid mass transfer, yielding biomass increases of approximately 30–45% across several biofuel-relevant microalgal strains as well as macroalgae species, whilst reducing CO<sub>2</sub> and mineral-nutrient inputs by 15–30%. These gains arise from enhanced gas residence time and diffusivity, rather than increased gas dosing. The approach is readily transferable between low-technology raceways and higher-specification photobioreactors, thereby reducing deployment risk.

Beyond algaculture per se, field trials demonstrate that nanobubble-enabled delivery of microalgal consortia to agricultural soils improves soil organic carbon, microbial biomass, aggregation, infiltration, crop yield, and water-use efficiency. Crucially, oxidative nanobubbles establish persistent redox control within soil-pore networks, accelerating pollutant degradation and reducing the bioavailability of salinity and heavy metals, independently of algal bioaccumulation. Taken together, these findings position electric-field NB-enhanced algaculture as a scalable, low-emissions platform linking sustainable biomass production with oxidative soil-bioremediation pathways of direct relevance to soil-health priorities.

## Introduction

Macro- and micro-algae are increasingly recognised as strategically important, low-emissions biological resources for food, feed, bio-based materials, and environmental remediation. Macro-algae (seaweeds), cultivated primarily in marine and coastal environments, and microalgae, grown in open-pond raceways or closed photobioreactor systems, both exhibit high areal productivity, rapid growth rates, and efficient CO<sub>2</sub> fixation relative to terrestrial crops, whilst avoiding direct competition for arable land and freshwater resources [1-4]. Collectively, macro- and micro-algae constitute a core pillar of emerging sustainable bioeconomy value chains, *inter alia* encompassing nutrition, agriculture, functional materials, pharmaceuticals, and

ecosystem restoration [2,4].

Microalgae are particularly attractive as sources of high-quality protein, polyunsaturated fatty acids, pigments, vitamins, and micro-nutrients, and may be cultivated using non-potable water streams, including saline water and municipal or industrial wastewater [5-7]. Macro-algae, by contrast, are already produced globally at multi-million-tonne scale and provide biomass suitable for food, feed, fertilisers, and soil conditioners, whilst simultaneously contributing to coastal nutrient removal and blue-carbon mitigation strategies [3,8]. In Europe, for instance, both macro- and micro-algal cultivation align closely with European priorities under the EU Bioeconomy Strategy and the European Green Deal, particularly with respect to, *inter alia*,



climate neutrality, food security, and circular resource use [9,10].

Notwithstanding this potential, large-scale algal cultivation in Europe remains constrained by significant economic and technological challenges. Amongst these, inefficient gas-liquid mass transfer-especially with respect to CO<sub>2</sub> supply and O<sub>2</sub> removal-remains a fundamental limitation in both open-pond and photobioreactor-based systems [11,12]. Conventional macrobubble aeration is characterised by rapid bubble rise, poor gas residence time, and substantial degassing losses, resulting in sub-optimal carbon-use efficiency and elevated energy demand, whilst closed systems are often limited by gas-dissolution kinetics and diffusional constraints [11]. Addressing these limitations is therefore essential to improving productivity, reducing operating costs, and lowering the life-cycle greenhouse-gas intensity of European algalculture.

Within this context, nanobubbles (NBs)-typically defined as gas bubbles with characteristic diameters below approximately 200nm-have attracted increasing attention due to their long lifetimes, elevated internal pressures, and exceptionally high specific interfacial areas [13,14]. Of particular relevance are electric-field-driven nanobubble-generation approaches, in which externally applied electric fields induce local electrostrictive and dielectric phenomena in liquids, thereby facilitating nanoscale gas entrapment without the use of chemical additives or high-shear mechanical processes. Recent work by English has provided a rigorous physical treatment of such mechanisms, situating them within the long-standing drive to elucidate nanobubble formation, metastability, and apparent deviations from equilibrium gas solubility under non-equilibrium electrostatic conditions, as compared to traditional mechanical-generation approaches [15].

Electric-field-generated nanobubbles offer distinct advantages for algal cultivation, insofar as they may increase the local availability and effective residence time of CO<sub>2</sub> and O<sub>2</sub> beyond conventional equilibrium solubility, whilst mitigating gas losses associated with buoyancy-driven detachment governed by Henry's and Stokes' laws [14-16]. Acting as quasi-reservoirs or buffers of bioavailable gas, nanobubbles can respond dynamically to photosynthetic demand, thereby improving carbon-use efficiency and biomass productivity in both micro- and macro-algal cultivation systems, without the need for increased gas injection per se [16,17].

Beyond biomass production in and of itself, integrated algae-nanobubble systems are of growing relevance to soil bioremediation, which has emerged as a strategic priority within Europe, for example, owing to widespread contamination by heavy metals, hydrocarbons, pesticides, and other legacy industrial pollutants [18-20]. Both micro- and macro-algae exhibit substantial biosorption and bioaccumulation capacities for metals such as cadmium, lead, copper, and zinc, and can contribute to the stabilisation, transformation, or partial degradation of organic contaminants [21,22]. In parallel, nanobubble-enriched water, particularly when oxygenated, has been demonstrated to enhance aerobic microbial metabolism, redox control, and degradation pathways within contaminated soils and sediments [23,24].

When deployed conjointly, algae and nanobubbles may act synergistically within soil systems. Nanobubbles enhance oxygen and carbon dioxide transport within soil pore water, stimulating microbial and algal metabolic activity, whilst algal biomass and exudates contribute organic matter, improve soil aggregation, and increase contaminant-binding capacity [22-25]. Moreover, macro-algal biomass - applied either directly or following processing into biochar or analogous soil amendments - can further enhance cation-exchange capacity and promote long-term sequestration of metals and organic pollutants [26]. Such integrated, nature-based approaches are closely aligned with international soil-health actions and restorative land-use strategies - offering scalable, low-emissions pathways for land rehabilita-

tion alongside sustainable biomass production [20].

Taken together, advances in electric-field-driven nanobubble technology, combined with macro- and micro-algal cultivation, represent a promising frontier for European bio-based innovation. These approaches address fundamental mass-transfer limitations in algalculture, reduce energy and carbon intensity, and extend the utility of algae beyond biomass generation towards multifunctional environmental remediation, thereby supporting Europe's transition to a resilient, resource-efficient, and climate-neutral bioeconomy.

## Methodology

In the present study, the electric-field NB-generation approach was employed [15], which has shown promising results for oxygen- and carbonation-transfer efficiency and gas-transfer rate [27]. The methodology of the industrial-scale continuous-flow NB generator with both air and CO<sub>2</sub> as the supply gas was used as in ref. 27, in addition to the submersible nanobubble generator [27] for select in-tank batch macro- and micro-algae cultivation under aeration conditions. As in ref. 27, these were supplied by AquaB Nanobubble Innovations Ltd.

In general, with the electric-field approach to NB generation, there is a rapid boost in the nanobubble population and enhanced NB diffusion, and the electrostatic circumstances of the nanobubbles' birth allows them to attract nutrients more easily to them, for, e.g., more efficient nutrient delivery in algalculture water though algae cell-wall matrices. This generation method results in a greater mass of gas to be accommodated beyond conventional Henry's-Law level (or "traditionally-dissolved" gas - i.e., as isolated molecules surrounded by solvent, e.g., water) on a metastable level - meaning that NBs can be released more easily when the time comes for their storage or use. The mass of gas in NB form is not strongly dependent on temperature, as Henry's-Law solubility is inherently - with this traditional solubility declining markedly at higher temperature (which explains why fish kills and blue-green algae are much worse in Summer time and hot weather, with lack of dissolved-oxygen solubility); this means that higher-temperature algae cultivation at 24-26 °C is boosted all the more by NBs. Importantly, these nanobubbles are kinetically metastable, typically for many weeks and longer, if there is little to no chemical or biological gas demand [15]. The energy costs of this additive-free, non-contact method achieve carbon-/gas-accommodation an order of magnitude higher than classical macro-/micro-bubble sparging, with superior nutrient transport (requiring less CO<sub>2</sub>, air/O<sub>2</sub>, nutrient, and overall energy/carbon footprint, and less maintenance) [15,27].

In any event, the electric-field's nano-gasification technology has many important distinctive advantages over existing microalgae-cultivation and soil-activity competitors, such as many and varied important desiderata:

- a. Very energy-efficient (about 35% of mechanical-generation cost); ideal for solar panels and Off-Grid;
- b. Species-selective formation of nanobubbles (e.g., 3-fold preferential CO<sub>2</sub> uptake from air, bio- and flue-gas);
- c. Retro-fit and increase capacity of footprint-constrained plant: reduce gasification energy by ~30%;
- d. Ability to transport additives (e.g., algae-feed mineral N-and P-nutrients, etc) by electrostatic adsorption; thereon - meaning that ~15-20% less mineral nutrient needs to be added to the liquid-gas mix!
- e. No moving parts, and so much less shear to allow cultivation of higher-value, shear-sensitive strains;
- f. Simple scale-up and continuous-flow operation, with serial or par-



allel operation simple;

g. Portable (e.g., deployment on floating island for solar-powered algae farming);

h. Excellent cleaning/sterilisation properties (e.g., virus-killing): ozone is 12 times longer-lived in water;

i. Works well at low and ambient pressure, or indeed at high pressures (tested up to 500 bar g);

j. Contact-free with water or process liquid: especially important for PBRs and “light-penetration” designs;

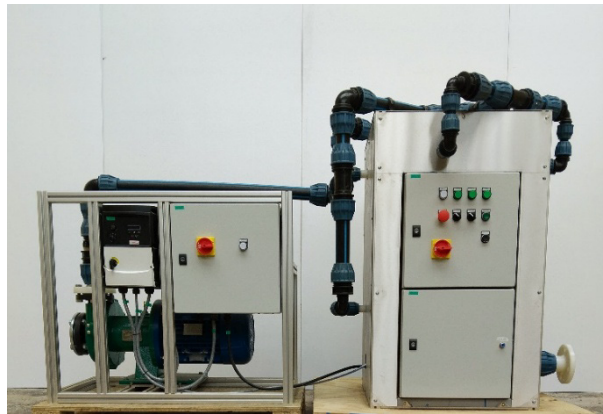
k. No membrane, so no problem of blockages (as happens with filter

or membrane-based CO<sub>2</sub>-capture systems)

l. Good level of quantifiability of CO<sub>2</sub> capture supporting “carbon accounting” (credit- and emissions-offsets, for potential block-chain integration): dissolved-CO<sub>2</sub> probes support 1mg/l resolution;

m. Few obvious or anticipated regulatory difficulties - yet still requiring project work in this area.

In terms of a larger-scale TRL-8 embodiment, we present this factory-floor photo in Figure 1. There are inlet and outlet pipes for the process liquid (not necessarily water), and the inlet gas can be air (with or without enhanced levels of CO, CO<sub>2</sub>, methane, etc) or flue gas, etc.



**Figure 1:** Side-views of one/multiple 1,000-l.p.m. water-flow model of “Vulcan-500” nanobubble generators, with inlet- and outlet-manifold piping and pump arrangements. Mass-flow controllers are installed for controlling of gas flow, with variable-speed drives on the water/aqueous pumps for liquid-flow control, and settings for ratio control of liquid to gas (and subsequent nanobubble formation).

## Results & Discussion

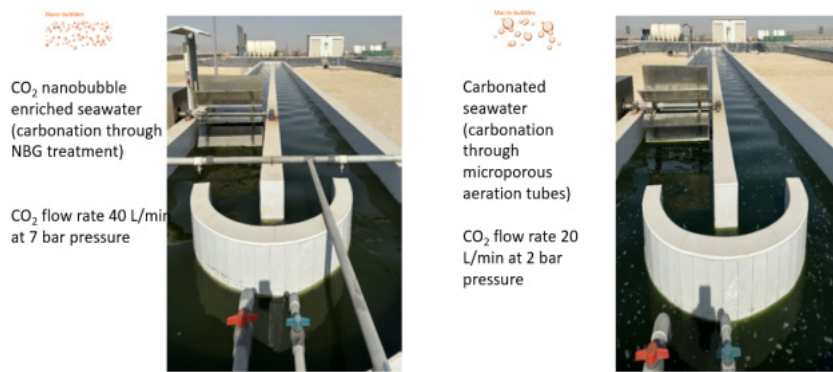
Marine-microalgae cultivation in a 25,000-litre freshwater raceway pond led to improved levels of biomass cultivation for *N. gaditana* (for biofuels) with both less CO<sub>2</sub> and less nutrient inputs. Figure 2 and Table 1 show the raceway-pond set-up and basic marine-consortium details for *N. gaditana*, respectively, for the control of standard CO<sub>2</sub> spargers versus inactivated spargers and use instead of “pre-nanocarobonated” seawater, whilst Figure 3 details impressively faster carbon-

ation kinetics for CO<sub>2</sub> nanobubbles. Table 2 summarises the findings of, inter alia, biomass yield up by a third, ~25% decline in CO<sub>2</sub> need and ~15% less mineral nutrients – leading to a ~30% OPEX reduction. Typically, within limits, there is a “trade-off” for one item (e.g., nutrient input) to lead to an improvement in the other two (e.g., biomass productivity and CO<sub>2</sub> provision). With electric-field nanobubbles, we remove from microalgae dynamics the “trade-off” aspect from these three “headline” variables, and also for carbon footprint.

**Table 1:** Basic experimental design, and high-level results (below in Table 2), for open-raceway-pond nanobubbles retrofit.

Details	RW101	RW102
Type of algae	Marine algal consortium	Marine algal consortium
Culture medium	Seawater	Seawater
Agitation	with paddle wheel	with paddle wheel
Carbon supplementation	CO <sub>2</sub> nanobubble enriched seawater using NBG	CO <sub>2</sub> sparging/bubbling using microporous tubes
Pond length (m)	34.5	34.5
Pond width (m)	3.5	3.5
Culture depth (m)	0.2	0.2
Working volume (m <sup>3</sup> /L)	24/24000	24/24000
Surface area (m <sup>2</sup> )	120	120
Harvesting	5000 L/day	5000 L/day
Avg. initial pond pH	7.32	7.61
Avg. final pond pH after adding carbonated seawater	6.66	6.82
Avg. CO <sub>2</sub> concentration (mg/L)	932	952



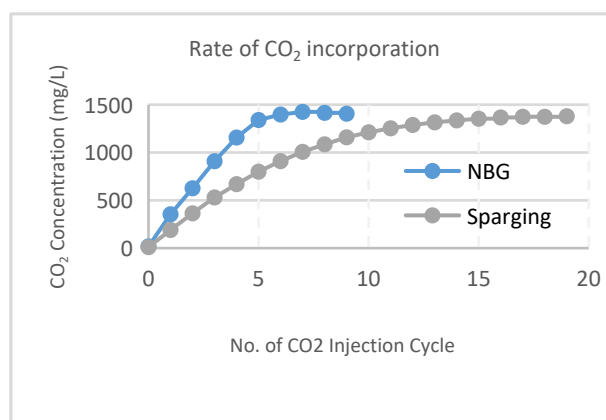


**Figure 2:**

(a) 200 l.p.m. flow of CO<sub>2</sub>-NB water through a retrofitted raceway pond for biofuels and algal water treatment.

(b) Same scenario without NBs, but with regular CO<sub>2</sub>-sparging, in which much “surface-frothing” is visible – showing rapid CO<sub>2</sub> loss by large bubbles rising (which is wasteful and greenhouse-gas-polluting).

Note the absence of frothing CO<sub>2</sub> escape for NBs. There is also better light penetration in the clearer NB pond-water (left).



**Figure 3:** Kinetics of CO<sub>2</sub> uptake by seawater *N. gaditana* cultivation, where nanobubbles from the NB generator (“NBG”) result in substantially faster CO<sub>2</sub> incorporation into the marine-culture medium vis-à-vis regular CO<sub>2</sub> sparging. In the CO<sub>2</sub>-nanobubbles case, this substantially faster mass-transfer kinetics and diffusivity of quickly-enhanced CO<sub>2</sub> concentration leads to the need to inject less CO<sub>2</sub> (cf. Table 2), whilst having less atmospheric escape (by avoiding fast-rising regular bubbles escaping at the frothy surface, as highlighted in Figure 2b). In terms of gas-transfer efficiency (i.e., percentage mass of gas dissolved in the partially-open-to-atmosphere liquid phase in the raceway as a proportion of that delivered), this was 98-99.5% in the first residence time, only declining to about 90% at Henry’s-Law (i.e., thermodynamic) saturation.

**Table 2:** High-level results from the comparison of nanobubbles (left) versus regular CO<sub>2</sub> sparging (right). The boost in biomass productivity combined with less CO<sub>2</sub> needed (and less CO<sub>2</sub> escape to atmosphere, as noted in Fig. 2a vs b) and less nutrition (i.e., “inputs”) removes the “trade-off” dynamic of microalgae production.

	RW 101	RW 102	
Average initial pH of the culture before harvest	7.75	8.07	
Average final pH of the culture after harvest and carbonation	6.87	7.13	
Average salinity (ppt) maintained in the ponds	110	110	
Average water temperature (°C)	30.1	31.4	
Average volume of pond volume harvested daily (L)	5000	5000	
Mode of CO <sub>2</sub> supply	CO <sub>2</sub> nanobubble enriched seawater	CO <sub>2</sub> bubbling through microporous tubes	
Average quantity of CO <sub>2</sub> supplied (g/day)	4540	4660	
Duration of study	10 days	10 days	
Average areal biomass productivity (g/m <sup>2</sup> /day)	22.1	16.5	
Biomass productivity (g/120 m <sup>2</sup> /day)	2123	1785	34 % increase
CO <sub>2</sub> utilization efficiency (g of CO <sub>2</sub> utilized/g of ash-free dry biomass produced)	1.9	2.4	25% less CO <sub>2</sub>
g of N supplied/g of ash-free dry biomass produced	0.3	0.36	
g of P supplied/g of ash-free dry biomass produced	0.012	0.014	15% less nutrition

Nanobubble ← → Regular Sparging



The electric-field NB-generation approach has made further progress with Vulcan-500 NB generators (not optimised, however, for microalgae cultivation, e.g., cf. Figure 3), with elevated gas-transfer performance with near-100% efficiency shown in Figure 4, to cultivate also other biofuel-promising strains beyond *N. gaditana*, such as *N. oculata* and *D. tertiolecta*, with respective biomass boosts of 35-45% and parallel ca. 20-30% reductions in CO<sub>2</sub> and nutrient requirements when infusing CO<sub>2</sub> NBs. All biomass boosts and input reductions passed single-tailed Student's t-tests at 99% for all three strains.

The electric-field approach has made further progress in retrofitting PBRs. Using a submersible nanobubble-generator design, field trials on retrofitting their relatively basic fed-batch PBR design in the upstream feedwater tank with air nanobubbles obtained very high sustained overall concentration of 2g/l (or 2,000 mg/l), with no further optimisation (albeit with the PBR as an early-commercial prototype; this showed a ~15% yield increase for *Arthrospira platensis*. Taken together with simply retro-fitting continuous-flow raceway designs and batch/fed-batch PBR set-ups for microalgae feed-water, the biomass productivity may be improved substantially with little effort. This shows the effectiveness of reducing larger-bubble CO<sub>2</sub>/O<sub>2</sub> escape, as well as more efficient and faster delivery of both gas and nutrients to the microalgae consortia through cell-wall matrices.

In terms of macro-algae cultivation, following encouraging air-NB usage in the case of the submersible NB generator for PBR retrofit, we also explored in-tank cultivation of *Halymenia* and *Caulerpa* strains, where we saw ca. 8 and 9% enhancement in growth for mean delta weight, but with roughly half the original standard deviation compared to the control case of no air NBs. Particular air-NB effects were seen in a profusion of filamentous *Ulva* (increased by 17%), notably

around the submersible unit (i.e., the green film on the walls in Figure 6): here, the markedly thin thallus results in greater surface area exposed to the air-NB-containing water, proportional to the biomass. All growth increases passed single-tailed Student's t-test at 90%.

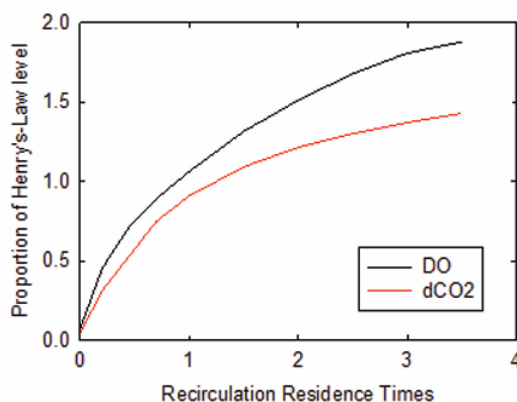
### Microalgae- and Nanobubble-Mediated Soil Regeneration

We undertook soil field trials at two contrasting sites:

a. sandy loam block with moderate organic matter depletion and nitrate-leaching risk, and

b. clay loam block with compaction, sodicity pockets, and reduced infiltration - to determine, in preliminary fashion, whether microalgae consortia delivered with oxygen/air NBs accelerate soil regeneration (e.g., organic-matter accrual, microbial activation, aggregate stability, etc) and remediation (e.g., reduced salinity and heavy metals), and support crop performance (yield, water-use efficiency).

Three strains were selected after NB-mediated cultivation in the manner described in part (a) above with non-algae-optimised Vulcan-500 NB generators (cf. Figure 1 & 5): *Chlorella vulgaris*, *Scenedesmus obliquus* and *Arthrospira platensis*. Consortia were maintained at 10<sup>5</sup>-10<sup>6</sup> cells/mL at the point of use in nanobubble water with dissolved-oxygen levels of at least 18mg/L. At the sandy- and clay-loam sites, the crops were, respectively, processing tomato and wheat. Treatments included grower standard, microalgae without nanobubbles (spray), and nanobubble-enabled microalgae (spray). Metrics included Soil Organic Carbon (SOC), Microbial Biomass Carbon (MBC), Electrical Conductivity (EC), aggregation, infiltration, yield, Water-Usage Efficiency (WUE), and energy.



**Figure 4:** Fixed gas-to-water flow ratios in a partially-open 3.5m<sup>3</sup> recirculation tank at a flowrate of 1 m<sup>3</sup>/min of water (i.e., a residence time of 3½mins). In water where the dissolved oxygen and CO<sub>2</sub> had been essentially removed by scavenging chemicals, the accumulation of dissolved gases was measured using a CboxQC meter (Anton Paar). It can be seen readily that supersaturated conditions were reached within about 1.5 residence times (about 8.5 and 1,400mg/l), and circa 90- and 45%-supersaturation plateaux within 3 such times (to ca. 16.5 & 2000mg/l, respectively).



**Figure 5:** A trailer-mounted Vulcan-500 NB generator on the left, alongside a quintet of such weather-shielded (IP-68) and skid-mounted generators connected to a flow manifold for mass oxygenation and carbonation of crop-spray and irrigation water for, inter alia, soil-regeneration trials, also using parallel administration of NB-cultivated microalgae.





**Figure 6:** Enhanced growth of *Ulva* species of macro-algae by air nanobubbles around the submersible NB generator – see the green film.

**Table 3:** % improvement compared to standard treatment in sandy-loam block, for *Chlorella vulgaris* and air NBs.

Clay-loam block, whilst using *Chlorella vulgaris* and air NBs, were as follows:

	Microalgae (regular water)	Nanobubble water	Microalgae & NBs
SOC	12	14	17
MBC	21	23	26
Aggregation	14	19	23
Infiltration	11	24	28
Tomato Yield	17	28	36
EC	17	21	26
WUE	11	14	16
Energy	8	22	26

**Table 4:** % improvement compared to standard treatment in clay-loam block, for *Chlorella vulgaris* and air NBs

The results for *Scenedesmus obliquus* are in Table 5 for both soil types in the case of air, and it is clear that the results are consistent.

	Microalgae (regular water)	Nanobubble water	Microalgae & NBs
SOC	10	12	15
MBC	20	22	23
Aggregation	11	17	21
Infiltration	9	22	24
Tomato Yield	16	25	32
EC	15	19	24
WUE	9	12	14
Energy	7	19	23

**Table 5:** % improvement compared to standard treatment in sandy- and clay-loam blocks, respectively, for *Scenedesmus obliquus* and air NBs.

The same is true of *Arthrospira platensis*, as is evident from Table 6:

	Microalgae (regular water)	Nanobubble water	Microalgae & NBs
SOC	10-Nov	13 / 12	17 / 16
MBC	17 / 15	20 / 19	24 / 22
Aggregation	9-Oct	16 / 15	20 / 18
Infiltration	8-Sep	20 / 19	23 / 22
Tomato Yield	15 / 14	23 / 21	29 / 27
EC	14 / 14	17 / 16	21 / 20
WUE	7-Aug	10-Nov	13 / 12
Energy	7-Jul	17 / 16	21 / 20



**Table 6:** % improvement compared to standard treatment in sandy- and clay-loam blocks, respectively, for *Arthrospira platensis* and air NBs

In the case of application of CO<sub>2</sub> instead of air NBs, it was found that there was a ca. 15% boost in growth vis-à-vis air, owing to stomatal penetration via spray delivery, and this is shown below in Table 7 for the case of the sandy-loam block, for *Chlorella vulgaris*:

	Microalgae (regular water)	Nanobubble water	Microalgae & NBs
SOC	10-Oct	14 / 13	16 / 15
MBC	18 / 17	21 / 18	23 / 21
Aggregation	8-Sep	17 / 16	21 / 19
Infiltration	9-Oct	21 / 20	21 / 22
Tomato Yield	14 / 15	22 / 22	28 / 26
EC	11-Dec	18 / 17	20 / 19
WUE	8-Sep	11-Dec	14 / 14
Energy	7-Jun	16 / 15	19 / 20

From these consistent results across microalgae type and NB gas in spray operation in both soil types, it can be seen that there is a positive synergistic interaction in a 22 factorial design, ANOVA-confirmed, with meaningful interaction effects present in all of the various dependent variables in the left column as a function of the independent variables (i.e., application of NBs and of microalgae). It was found microalgae use and NBs was somewhat more effective in sandy- than clay-loam-block, owing, in part, to soil porosity versus surface-tension reductions in NB water being offering more hydrological responsiveness in sandy-loam block, as well as concomitant effects on promoting greater microbiological diversity and health from easier matrix penetration.

The simplest and lowest-OPEX operations, in terms of using both air and *Chlorella vulgaris*; is broadly true of the other two algae types and also CO<sub>2</sub>, given stomatal penetration into crops by CO<sub>2</sub> NBs in spray configurations (as suggested in earlier field-trial designs [27]). It was also seen that results could be improved somewhat using some of these other gas/algae-strain combinations, although full statistical confirmation by F-test metrics does not (yet) quite reach the 95% threshold.

**Abiotic Soil Redox Dynamics from Nanobubbles**

Importantly, the field trials demonstrate that the soil-regeneration effects observed are not explained by biological inputs alone, but by a sustained nanobubble-induced modification of soil redox dynamics. Delivered oxygen/air nanobubbles provide a persistent oxidative capacity within the soil matrix, which enhances degradation kinetics of redox-sensitive organic contaminants and drives heavy metals towards less mobile and less bioavailable oxidation states through mineral association and aggregate binding.

In this system, microalgae are not relied upon as sequestration sinks for contaminants. Any transient biological interaction is secondary to the nanobubble-mediated redox control that remains active post-application, including within applied biomass itself. As a consequence, the parallel application of microalgae and nanobubbles to soil avoids contaminant transfer risks commonly associated with bioaccumulation-based remediation.

Importantly, soil-applied biomass is exclusively derived from defined, monitored nutrient streams. Biomass exposed to elevated or persistent contaminants is excluded from soil use, and remediation objectives are assessed using reductions in bioavailable fractions (e.g. EC, plant-available metals), not total contaminant mass. These mechanisms explain the observed decreases in soil salinity, improved aggregation, infiltration, and microbial activity measured across field sites.

**Soil Redox Dynamics and Oxygen Persistence**

Across both sandy- and clay-loam sites, treatments receiving nano-

bubble enriched irrigation water (T2: nanobubbles only; T3: nanobubbles and microalgae) exhibited a sustained elevation in soil redox potential (Eh) relative to both the grower standard (T0) and microalgae-only treatment (T1). Mean Eh values at 10–30cm depth increased by ~140–160 mV under nanobubble treatments and remained within an oxidative régime for at least seven days following application, whereas T0 and T1 returned to near baseline redox conditions within 48–72 hours.

Dissolved-oxygen concentrations in soil pore water remained at 6-9 mg L<sup>-1</sup> at 72 hrs following application in T2 and T3, compared with <2 mg L<sup>-1</sup> in T0 and T1. The temporal stability of Eh ( $\Delta Eh < \pm 20$  mV over 7 days) in nanobubble-amended soils indicates that observed effects arise primarily from a persistent physicochemical modification of the soil redox environment rather than from transient biological respiration alone.

**Degradation of Redox-Sensitive Organic Contaminants**

Soils containing moderate petroleum hydrocarbon contamination showed significantly accelerated degradation under nanobubble treatments. After 14 days, total extractable hydrocarbons were reduced by ~18% (T2) and ~24% (T3), compared with only 3% in the control and 11% under microalgae alone. The consistent improvement observed for T3 vis-à-vis T2 suggests that algal consortia augment remediation indirectly – most probably through microbial stimulation - whilst the dominant mechanism remains nanobubble-driven oxidative redox control.

**Heavy-Metal Bioavailability and Speciation**

The total concentrations of Pb and Cd remained unchanged across treatments, confirming that remediation was not attributable to contaminant removal per se. However, DTPA-extractable (i.e., plant-available) fractions declined substantially under nanobubble treatments, with bioavailable Pb reduced by 35–50% and Cd by 30–45% vis-à-vis the control. These reductions are consistent with oxidation-state stabilisation and enhanced mineral association under persistently oxidative conditions, as opposed to biological sequestration.

**Soil Structure, Salinity, and Microbial Activity**

Nanobubble-amended soils exhibited marked improvements in, inter alia, aggregation, infiltration, soil organic carbon (SOC), and microbial biomass carbon (MBC), alongside a consistent reduction in electrical conductivity (EC). Improvements were more pronounced in sandy-loam soils, reflecting the greater responsiveness to surface tension reduction and enhanced pore-scale oxygen transport. Microalgae alone produced modest gains, yet these were amplified substantially when combined with nanobubbles, indicating a synergistic – albeit redox-dominated - interaction.



### Oxidative Degradation of Organic Pollutants within Applied Algal Biomass

To assess whether applied algal biomass could act as a secondary reservoir for organic contaminants, hydrocarbons and representative pesticide residues were quantified within algal biomass recovered from soil surfaces and upper pore layers at Days 0, 7, and 14 following application. The organic contaminants in the applied biomass were as follows, in terms of C<sub>10</sub>-C<sub>40</sub> hydrocarbon equivalents:

In nanobubbleenabled treatments (T3), organic contaminant loads within biomass declined by ~70% within 14 days, whereas only minimal reductions were observed for microalgae alone. This indicates that algal biomass does not function as a longterm contaminant sink and that nanobubblemediated oxidative processes remain active within and around the biomass matrix postapplication. This suggests a mechanistic interpretation that air nanobubbles generate reactive oxygen species (ROS) [15], including •OH and O<sub>2</sub>•<sup>-</sup>, through interfacial collapse and chargedensity effects at the gas-liquid interface. These ROS promote oxidative scission of adsorbed hydrocarbons and pesticide residues, as well as breakdown of polymeric cellwallassociated organic matter. This also facilitates the prevention of contaminant rerelease during biomass decay – an important characteristic. As a result, organic pollutants associated with applied biomass are mineralised progressively, rather than redistributed within the soil system.

### RedoxDriven Metal Bioremediation via NanobubbleDerived Reactive Species

Speciation analysis was conducted for redoxsensitive metals to distinguish between biological uptake and physicochemical transformation pathways. In the case of chromium, the Cr(VI) → Cr(III) was studied:

It can be seen from Table 9 that nanobubble treatments drive a pronounced shift from soluble, toxic Cr(VI) towards sparingly soluble Cr(III) - confirming oxidative-reductive speciation control rather than uptake. Total chromium remained constant, ruling out sequestration or removal per se.

À propos copper and iron redox cycling, this was also studied for the T0 and T3 treatments, and the results summarised below in Table 10:

Once more, Table 10 shows that it is readily evident that nanobubblederived oxidative species accelerate conversion of metals into oxidebound and residual fractions via enhanced surface complexation, coprecipitation, and aggregate incorporation. These fractions exhibit markedly lower mobility and bio-availability. Metal bioremediation is dominated by (abiotic) nanobubble effects. The interaction term indicates that organic ligands and surfaces introduced by algae certainly facilitate – albeit, ceteris paribus, do not drive - the oxidative transformation and immobilisation process.

**Table 7:** % improvement compared to standard treatment in sandy-loam block, for *Chlorella vulgaris* and CO<sub>2</sub> NBs.

	Microalgae (regular water)	Nanobubble water	Microalgae & NBs
SOC	14	16	19
MBC	23	26	29
Aggregation	16	21	26
Infiltration	13	26	32
Tomato Yield	19	30	38
EC	18	23	29
WUE	13	16	18
Energy	9	25	29

**Table 8:** Organic contaminants in applied biomass.

Treatment	Day 0 (mgkg <sup>-1</sup> dry biomass)	Day 7	Day 14
T1 – Microalgae only	62	58	56
T3 – Microalgae and NBs	61	34	21

**Table 9:** Cr (VI) → Cr (III), and bio-availability.

Treatment	Cr (VI) (% of total Cr)	Cr (III) (% of total Cr)	Bio-available Cr (%)
T0	38	62	29
T1	34	66	26
T2	18	82	14
T3	12	88	9

**Table 10:** Parameters for copper and iron redox cycling.

Metal	Treatment	Exchangeable (%)	Oxide Bound (%)	Residual (%)
Cu	T0	27	41	32
Cu	T3	11	59	30
Fe	T0	19	46	35
Fe	T3	7	63	30



## Conclusions

Prototype trials of electric-field-driven nanobubble (NB) generation with particular emphasis on nano-carbonation have been applied to micro- and macro-algal cultivation systems and subsequently to soil regeneration. In raceway-pond and retrofitted photobioreactor configurations, nano-aeration and nano-carbonation improved gas-liquid mass transfer, yielding biomass increases of approximately 30–45% across several biofuel-relevant microalgal strains as well as macroalgae species, whilst reducing CO<sub>2</sub> and mineral-nutrient inputs by 15–30%.

Nanobubble-enabled delivery of microalgal consortia to agricultural soils improves soil organic carbon, microbial biomass, aggregation, infiltration, crop yield, and water-use efficiency. Fieldtrial data demonstrate that nanobubble-derived oxidative reactive species exert sustained control over both organic and inorganic contaminants. Organic pollutants associated with applied algal biomass are progressively degraded rather than sequestered, whilst heavy metals undergo redox-driven transformation into less mobile and less bioavailable forms. These effects persist independently of algal metabolism and remain active following biomass turnover, thereby minimising contaminant transfer risk and distinguishing this approach fundamentally from bioaccumulation-based remediation strategies.

Taken together, these findings position electric-field NB-enhanced algalculture as a scalable, low-emissions platform linking sustainable biomass production with oxidative soil-bioremediation pathways of direct relevance to soil-health priorities.

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