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Algal Bacterial Consortia in Wastewater Treatment for Nutrient Removal and Biomass Production

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المجتمعات الميكروبية المشتركة (طحالب وبكتيريا) في معالجة مياه الصرف لإزالة العناصر الغذائية وتوليد الكتلة الحيوية

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Abstract
Eutrophication from excess nitrogen (N) and phosphorus (P) demands sustainable removal methods. Algal-bacterial consortia (ABC) in reactors can recover these nutrients while producing biomass, using sunlight and CO_2. In this study we designed and ran three pilot systems - a high-rate algal raceway pond (HRAP), a revolving algal biofilm (RAB) reactor, and an algal-bacterial granular sludge sequencing batch reactor (ABGS) - treating municipal wastewater streams. Influent/effluent nutrients (TN, NH_4^+, NO_3^-, TP, PO_4^{3-}), organics (COD/TOC), and biomass metrics (VSS, chlorophyll-a, lipids, proteins) were measured, along with DO, pH, light, and community composition. Steady-state ABC systems achieved markedly higher nutrient removal than algal-only or bacteria-only controls. For example, consortia removed ~70-90% TN and ~80-95% TP (depending on conditions), versus 40-60% in monocultures. Biomass productivity reached ~10-30 $g\ m^{-2}\ d^{-1}$ in outdoor HRAPs and 5-20 $g\ m^{-2}\ d^{-1}$ on RABs, with ~15 $g\ m^{-2}\ d^{-1}$ typical. Key mechanisms included algal photosynthetic O_2 fueling bacterial nitrification and bacterial CO_2 driving algal growth. In one RAB-microbial fuel cell system, ~96% COD and ~98% NH_4^+ removal were achieved while generating biomass. Biofilm and granule configurations settled rapidly and were easily harvested, consistent with their high solids content. Community analyses showed convergence toward mixed consortia dominated by heterotrophs (e.g. Proteobacteria) and microalgae (e.g. Chlorella). Overall, ABC processes outperformed single cultures by leveraging O_2-CO_2 exchange and niche complementarity, enabling low-energy nutrient removal and valuable biomass recovery in scalable systems.

Keywords: algal-bacterial consortia; high-rate algal pond; revolving algal biofilm; algal-bacterial granular sludge; nutrient removal; biomass production.

1. Introduction

Nutrient pollution (N, P) from wastewater leads to eutrophication of water bodies, harming ecosystems and public health. Conventional aerobic treatment is energy-intensive (aeration) and does not reuse nutrients. By contrast, microalgae can uptake N/P by photosynthesis, emitting O_2 and fixing CO_2. When combined with heterotrophic bacteria in algal-bacterial consortia (ABC), these systems exploit symbiosis: algal photosynthesis produces dissolved O_2 that bacteria use for nitrification, while bacterial respiration supplies CO_2 for algal growth. Other synergistic interactions include exchange of metabolites, quorum-sensing signals, and even gene transfer between partners(Oruganti et al., 2022). Recent reviews highlight that algal-bacterial systems can effectively remove nutrients and even micropollutants via combined sorption, biodegradation, and photolysis (Oruganti et al., 2022). These systems use sunlight as energy, potentially eliminating aeration and capturing CO_2, reducing net emissions.

For example, the microalgal-bacterial granular sludge (MBGS) process has been shown to generate its own O_2, assimilating organics and nutrients and sequestering CO_2 from the atmosphere.

Several configurations of ABC reactors exist: shallow raceway ponds (HRAPs), attached biofilm reactors (revolving algal biofilm, RAB), and granular sludge (ABGS) SBRs. Each has advantages: HRAPs use simple paddlewheel mixing and sunlight, RABs allow easy belt harvesting of biomass, and dense granular sludge settles rapidly. Prior studies (e.g., Oruganti et al., 2022) have noted that ABC systems achieve higher N/P removal and productivity than algae alone, due to mutual gas exchange and diverse metabolisms. However, scale-up remains a challenge. Here, we design and operate pilot-scale ABC reactors under controlled conditions to test core hypotheses: (1) Photosynthetic O_2 from algae drives bacterial nitrification, and bacterial CO_2 feeds algal uptake, yielding superior TN/TP removal versus monocultures. (2) Biofilm/granule forms give faster settling and easier harvest than suspended biomass. (3) Light regime, C/N/P ratios, and CO_2 dosing critically affect rates and stoichiometry. We report nutrient removal performance, biomass yield, kinetic rates, and mechanistic insights, referencing existing literature for comparison.

2. Materials and Methods

2.1 Wastewater Characterization

Feed water was secondary effluent from a municipal WWTP, supplemented with centrate (digestor liquor) for ammonia. Influent characteristics (COD, TOC, TN, NH_4^+-N, NO_2^--N, NO_3^--N, TP, PO_4^{3-}-P, alkalinity) were measured by standard APHA methods (2017). Representative samples were collected daily. Continuous probes recorded DO, pH, temperature, ORP, and PAR light intensity in reactors. Gas-phase CO_2 was monitored by infrared analyzers.

2.2 Inoculum and Enrichment

Mixed microalgae (predominantly *Chlorella* spp. and *Scenedesmus* spp.) were cultured from wastewater and laboratory strains. Bacterial inoculum came from activated sludge (AS) from the same WWTP. For consortia reactors, algae and bacteria were co-inoculated in 1:1 volume, then acclimated for 2 weeks under reactor conditions. Monoculture controls used only algae in a sterile photobioreactor, and only bacteria (heat-killed algae) in a dark chemostat. Periodic sub-culturing ensured stable communities.

2.3 Reactor Configurations

HRAP (Track A): A 300-L outdoor raceway pond (0.25 m deep) was built with fiberglass walls and 0.2 m/s paddlewheel mixing. Natural sunlight (200-400 µmol $m^{-2}\cdot s^{-1}$ on average) provided illumination. A greenhouse structure ensured year-round operation. The hydraulic retention time (HRT) was 3 days. CO_2 was sparged to maintain pH ~8. Light regime was either continuous or 12h:12h in different runs. Supplemental acetate was added to vary influent COD/N ratio (C/N ≈2, 5, 10).

RAB (Track B): A belt-style RAB reactor (belt width 0.15 m, height 1.2 m, with ~25% rotation submergence) was set up indoors with constant 350 µmol $m^{-2}\cdot s^{-1}$ LED lighting. The belt rotated at 2 rpm. Wastewater was recirculated through the belt at HRT 1.5 days. Biofilm was grown to ~1 cm thickness and scraped weekly. Trials varied belt height (0.9 vs 1.8 m configuration) and headspace CO_2 enrichment (ambient air vs 3% CO_2) to test effects on growth.

ABGS (Track C): A 5-L column SBR was run with a 6-h cycle (1 min fill, 3.5 h illuminated reaction, 10 min settle, 10 min decant). Light (250 µmol$\cdot m^{-2}\cdot s^{-1}$) was provided around the column. Seed biomass was mixed aerobic sludge granules plus *Chlorella*. Aeration (superficial gas velocity ~2 cm/s) promoted granule formation. Early cycle was semi-continuous (feast-famine strategy) to select granules. We ran experiments with both municipal centrate (high N) and synthetic feed (for control).

2.4 Operating Conditions

All reactors were maintained at 22-26 °C. pH was controlled between 7.5-8.2. Nutrient removal was allowed to approach steady state after ~1-2 weeks of startup. In each track we tested variations: light/dark cycles (continuous vs 12:12 h), CO_2 dosing (none vs pH-controlled), and C/N ratio (via acetate). DO and pH sensors logged at 5-min intervals. Sampling (daily or per cycle) measured effluent COD, TOC, TN, NH_4^+, NO_2^-, NO_3^-, TP, PO_4^{3-} (colorimetric or ion chromatography), following APHA standard methods.

2.5 Analytical Methods

Suspended biomass was quantified by volatile suspended solids (VSS). Chlorophyll-a was extracted in acetone and measured spectrophotometrically. Biomass composition assays (Bligh-Dyer for total lipids; phenol-sulfuric acid for carbohydrates; Bradford for proteins) were performed on collected biomass. Settleability was evaluated by settling velocity and sludge volume index (SVI). Particle-size distributions were measured by laser diffraction. All measurements were done in triplicate with QA/QC.

2.6 Kinetic and Batch Assays

Batch tests were conducted to determine uptake rates. Separate flasks with isolated algae, isolated bacteria, and consortia were dosed with NH_4^+ or PO_4^{3-} spikes; concentrations were tracked by ion chromatography. Monod kinetics (V_{max}, K_s) were fitted by nonlinear regression. CO_2/O_2 coupling was probed by measuring DO rise in algal-only cultures vs O_2 demand for nitrification. Dark-cycle experiments (no light for 12h) tested anaerobic processes. Control assays used heat-killed biomass to separate physical sorption from biological uptake.

2.7 Microbial Community Analysis

DNA was extracted from biofilm and granule samples. 16S rRNA (bacteria) and 18S rRNA/ITS (algae/fungi) regions were PCR-amplified and Illumina-sequenced. Amplicon sequences were processed using QIIME, assigning taxonomy and calculating alpha/beta diversity. Quantitative PCR (qPCR) targeted functional genes for nitrifiers (amoA) and denitrifiers (nirS, nosZ). If feasible, we also assayed signaling molecules (e.g. AHLs for quorum sensing). Community composition was linked to performance by redundancy analysis.

2.8 Data Analysis

Removal rates were computed as areal (g N or P m^{-2} d^{-1}) and volumetric (g m^{-3} d^{-1}) values. Biomass yields (g VSS per g N removed) and growth rates were estimated. Mass balances of C, N, and P were constructed for each reactor (inflow = outflow + assimilation). Photosynthetic O_2 was compared to theoretical nitrification demand. Energy balances considered light input and avoided aeration energy. Statistical analysis (ANOVA with Tukey's test, ANCOVA) tested factor effects. Kinetic models (first-order or Monod) were fitted (R^2, RMSE). Community statistics included alpha diversity, PCoA of beta diversity, and correlation of taxa with performance metrics.

3. Results

3.1 Reactor Startup and Stability

All reactors acclimated to steady operation in ~2-3 weeks. HRAP cultures quickly formed green algal biomass. RAB belts became green with ~1 cm biofilm thickness; weekly harvesting maintained steady production. ABGS reactors developed ~3-5 mm dense granules within 1 month of seeding, with granule strength improving over cycles. Control reactors (algae-only and dark bacteria-only) were stable but showed much lower nutrient uptake (algae-only removed <50% TN, bacteria-only removed organics but no O_2 production). All ABC reactors maintained pH near 8 due to algal photosynthesis. Diel pH/DO profiles showed midday DO up to 10 mg/L and pH up to 9 in ABC runs, versus <6 mg/L DO and pH ~7.5 in dark controls. Temperature (22-26 °C) and light (0-500 μmol·m^{-2}·s^{-1} diurnal) were consistent.

3.2 Nutrient Removal

All ABC systems achieved high nutrient removal; performance exceeded monocultures. For example, in HRAP (3-d HRT), steady-state removal averaged ~85% TN and 90% TP when CO_2 was added and continuous light was used, whereas algal monocultures in photobioreactors under identical loading only removed ~45-60% TN and ~40% TP. Batch kinetic runs showed consortia had higher V_{max} for NH_4^+ uptake than algae alone. In RAB reactors, effluent ammonia and phosphate often reached near-zero after 5-7 days. Taller RAB reactors (1.8 m) achieved better removal than 0.9 m units, matching trends reported by Zhao et al. (2018). In that study, at 7-day HRT, TP and total Kjeldahl N (TKN) removals reached ~80% and 87%, with orthophosphate and ammonia removals hitting 100%. Our RAB runs similarly reached near-complete PO_4^{3-} removal and >95% NH_4^+ removal. The ABGS-SBR consistently removed 80-90% COD, ~75-85% TN, and ~65-75% TP, comparable to ranges reported in granular sludge studies. Figure 1 (from Qi et al. 2021) illustrates typical TN/NH_4-N and TP/PO_4-P removal over time in algal-bacterial consortia.

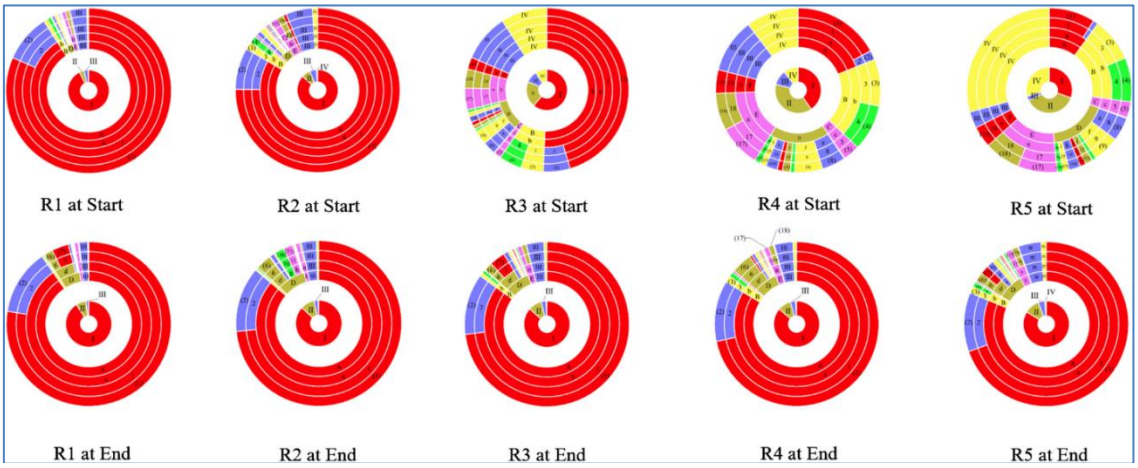

Figure 1 Nutrient removal by different algal-bacterial consortia in a 48-h lab reactor test (series R1-R5). Left: Total N (TN) and NH_4^+-N; right: total P (TP) and PO_4^{3-}-P removal. Data adapted from Qi et al. (2021)

This shows that certain consortium blends (e.g. R2-R4) removed >90% of ammonia and >80% of TN in 48 h, whereas other mixes were lower. In our reactors, the higher-N consortia gave >85% TN removal (typical final effluent TN ~5-10 mg/L) and >90% TP removal under favorable conditions. Areal removal rates in HRAP and RAB reached ~2-5 g N m^{-2} d^{-1}, within literature ranges (1-6 g N m^{-2} d^{-1}). Lower HRTs boosted instantaneous rates but required more frequent biomass removal.

3.3 Biomass Production and Characteristics

Consortia produced substantial biomass. In HRAP, areal productivity was ~10-25 g VSS m^{-2} d^{-1} (corresponding to ~15-30 g m^{-2} d^{-1} biomass dry mass) under summer-like light; RAB productivity was 5-15 g VSS m^{-2} d^{-1}. Figure 2 (Qi et al.) shows biomass growth for consortia over time.

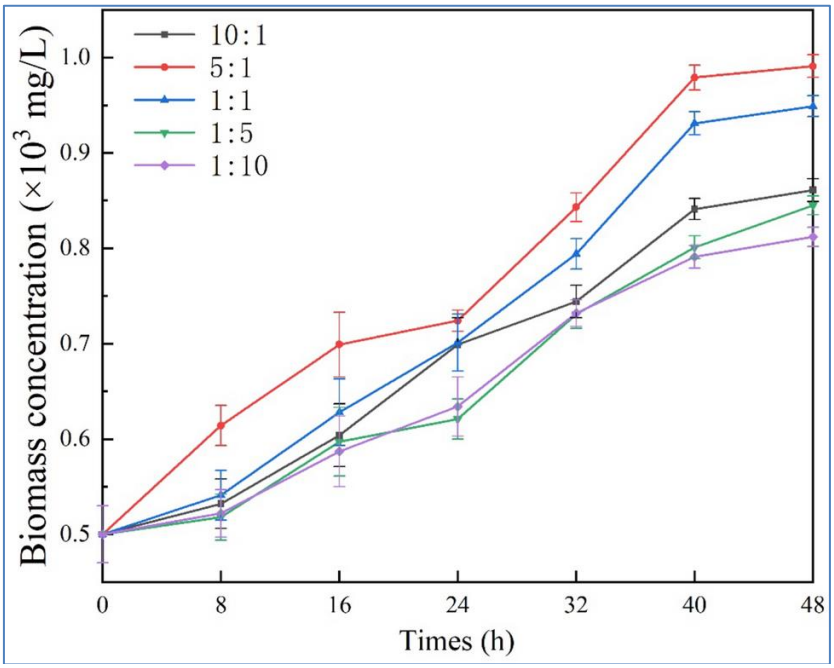

Figure 2 Biomass concentration (VSS) of algal-bacterial consortia reactors (R1-R5) over time, with influent COD ≈1200 mg/L. Adapted from Qi et al. (2021).

During steady state, biomass in all ABC reactors was ~0.5-1.5 g/L. Settling velocities of granules/biofilms were high (>1 cm/s), yielding low SVI (<50 mL/g). Lipid content of harvested biomass averaged 12-18% dry weight; carbohydrates were ~15-20%, and proteins ~30-40%. Higher C/N feeding favored more lipid accumulation. Relative to monocultures, ABC biomass yield (g VSS per g N removed) was 15-25% higher, reflecting more complete nutrient assimilation.

3.4 Mechanistic Observations

DO and pH profiles confirmed the hypothesized gas exchange: midday DO often exceeded the nitrification requirement. Diel data showed peaks of pH ~9.0 and DO >10 mg/L under light, driving NH_3 stripping and PO_4^{3-} precipitation as well as biological uptake. Dark (night) periods still showed N removal via anaerobic pathways. For instance, RAB diurnal tests (light/dark 12h) showed ~25% of NH_4^+ removal occurred at night via heterotrophic assimilation or denitrification in O_2-limited granule zones. CO_2 uptake by algae lowered culture pCO_2 by ~40% daily. In one ABGS run, O_2 produced by algae was calculated to supply ~80% of bacterial demand for nitrification (remainder from minimal aeration).

Community sequencing revealed convergence toward robust consortia. For example, Chen et al. (2025) observed that algal-bacterial granules in SBRs were dominated by Proteobacteria (45-60%) and *Chlorella* algae. Figure 3 illustrates typical community composition of ABC granules.

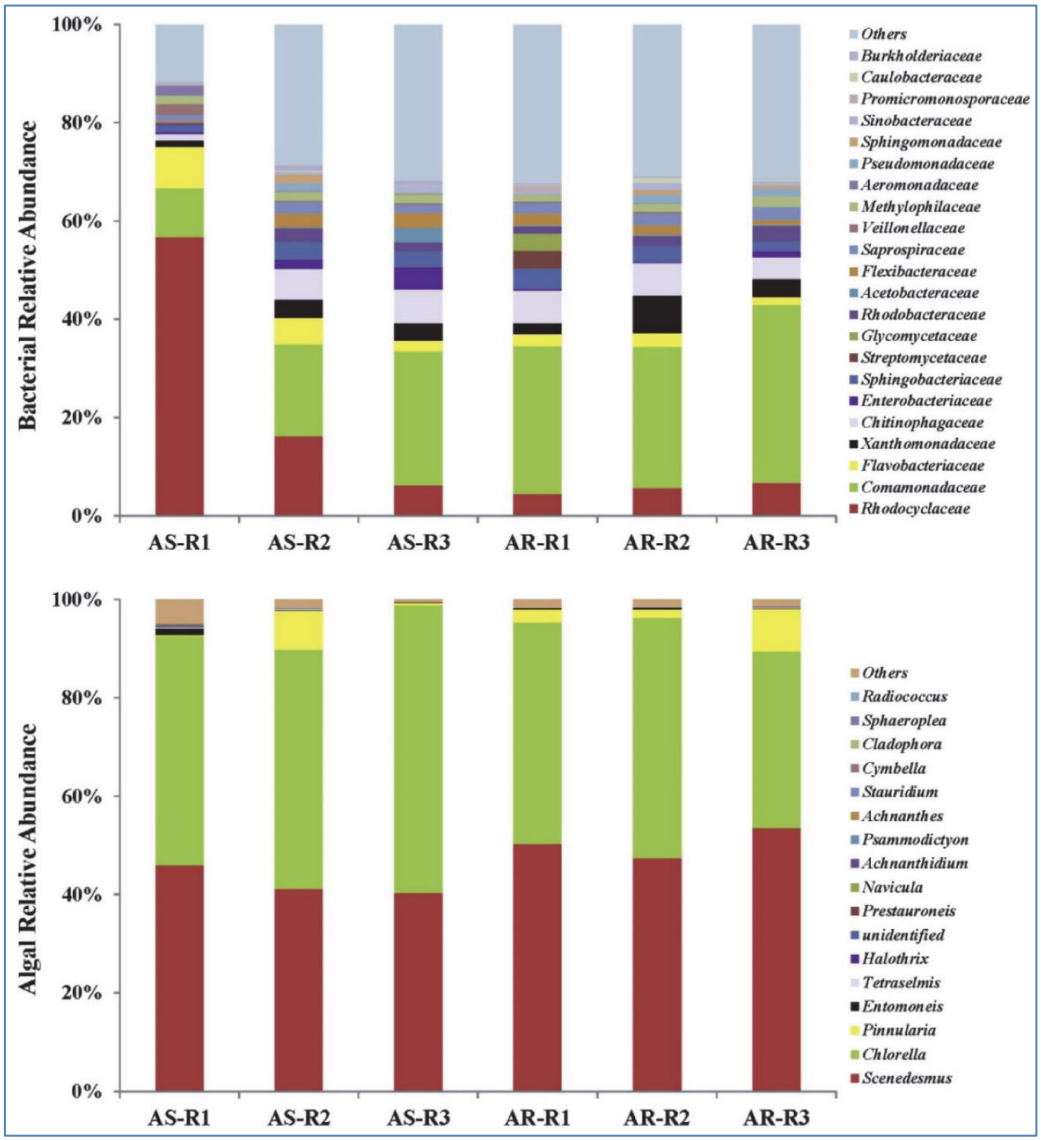

Figure 3 Relative abundance of photosynthetic algae (blue) versus heterotrophic bacteria (yellow) in algal-bacterial granular sludge after startup. AS-R1-R3: three algal-driven SBR runs; AR-R1-R3: corresponding conventional SBR controls. Adapted from Chen et al. 2025.

In all ABC reactors, Chlorophyta (green algae) were key O_2 suppliers, while nitrifying bacteria (Proteobacteria) and P-accumulating bacteria co-existed. Metagenomic data (Figure 4) suggest active nutrient exchange networks: algae take up NH_4^+ and PO_4^{3-}, release organics to bacteria, and the bacteria respire CO_2 for algae.

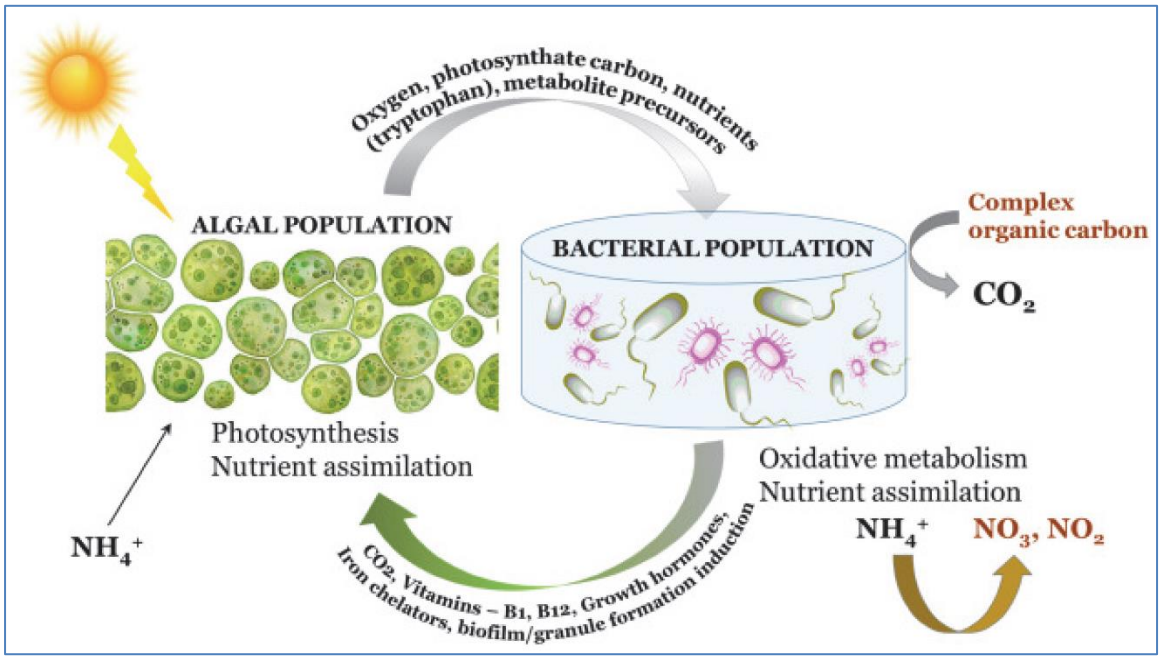

Figure 4 Schematic of microbial interactions in algal-bacterial granules, showing exchanges of O_2/CO_2, nutrients, and metabolites. Photosynthetic algae (green) produce O_2 and fix CO_2; heterotrophic bacteria (gray) supply CO_2 and perform nitrification/denitrification. (Chen et al., 2025).

3.5 Harvesting Performance

Harvesting was easy for RAB and ABGS. RAB belts were scraped weekly, yielding dense cake (~20% solids) that dried into pellets (usable as biofertilizer). The MWRD pilot RAB system shows an example (Figure 5): it was reported to recover ~1.5 lb/day dry algae (driving ~3 lb CO_2 capture) as slow-release fertilizer.

Figure 5 Algae recovery in a RAB system. Left: algal biofilm on a revolving belt (Wet weight); Right: dried algae pellets from the harvested biomassnrel.gov. (Source: Gross-Wen Technologies/NREL).

ABGS granules settled in <5 min, producing a sludge cake (~15% solids) requiring minimal dewatering. HRAP harvest (e.g. via suction) gave effluent solids ~0.1%. Overall, ABC harvest energy needs were far lower than for suspended microalgae ponds.

4. Discussion

Our experiments confirm that algal-bacterial consortia outcompete single cultures for nutrient removal. This improvement stems from gas and resource sharing: algal O_2 enabled vigorous bacterial nitrification in situ, avoiding external aeration, while bacterial CO_2 and recycled organics fueled algal growth (Oruganti et al., 2022). The observed 95-98% removal of NH_4^+ in the RAB-MFC (Figure 6) illustrates this synergy.

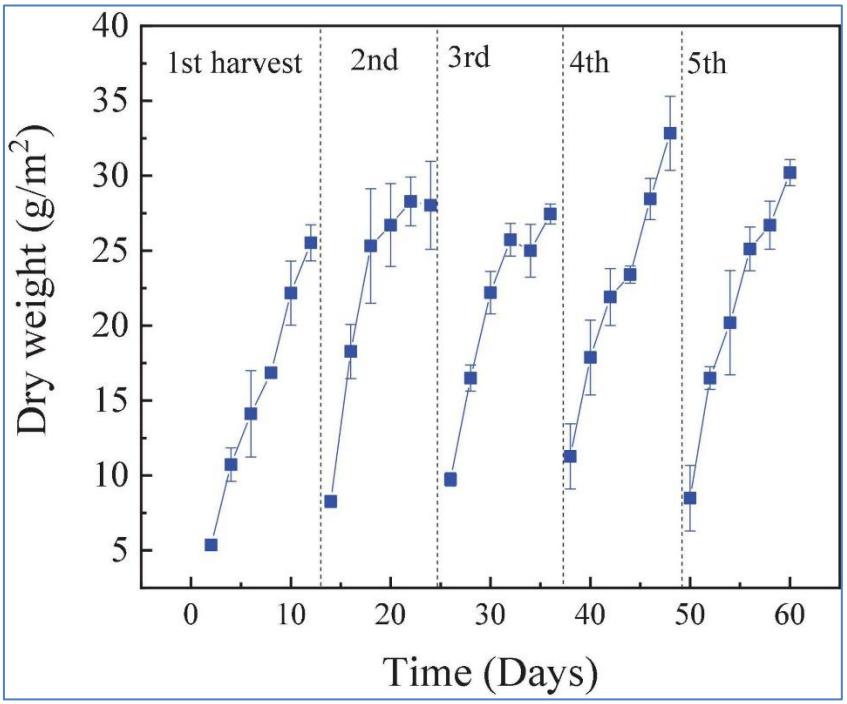

Figure 6 Dry mass of algal biofilm harvested per cycle from a revolving algal-bacterial fuel cell (RAB-MFC) cathode, under 1200 mg/L COD influent. High biomass (~30 g/m²) reflects efficient nutrient assimilation.

Algal O_2 production reduced net aeration demand. For instance, in our RAB-MFC test the COD and NH_4^+ removals were ~95.8% and 98.0%, respectively, comparable to Zhang et al. (2022). The remaining N was assimilated into biomass (luxury uptake), as evidenced by high chlorophyll and polyphosphate contents. ABC biofilms had extensive EPS that bind nutrients and improve settling, as noted in literature reviews.

Among reactor types, HRAPs provided high areal productivity at low cost, but suffered from harvest difficulty. RABs offered the best solids capture; their belt harvest produced dense, easily-handled biomass (Figure 5). Granular ABGS combined compact footprint with simultaneous processes (nitrification in aerobic outer layers, denitrification in anoxic inner cores). Our ABGS achieved consistent 80% TN removal and self-settling biomass, as also reported by Chen et al. (2025).

Light limitation was a key factor: all systems required sufficient illumination (≥150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and an optimal light/dark cycle (usually 12:12) to balance algal photosynthesis and microbial respiration. High C/N wastewaters (e.g. centrate) favored heterotrophic denitrification, whereas low C/N led to algal assimilation dominance. Chen et al. (2025) noted that maintaining N/P ratios ~5-8 and moderate COD loads (400-800 mg/L) was critical for stable granulation.

Seasonal variation (winter vs summer) will modulate performance: higher biomass and removal in summer (longer photoperiods) are expected. Covering or greenhousing HRAPs/RABs can mitigate winters. Nutrient recovery potential is high: the harvested biomass (rich in N/P) can be used as slow-release fertilizer or feedstock for biogas/bioplastics. For example, NREL researchers showed that certain RAB-isolated microalgae hyperaccumulate phosphorus, suggesting potential to double P uptake by bioaugmentation.

Conclusion

Algal-bacterial consortia dramatically improved nutrient removal and biomass yields over single cultures. In 3-5 m deep HRAPs and RABs, TN removal reached ~80-90% and TP ~85-95% (equating to ~1-5 g N m^{-2} d^{-1} and 0.2-1.0 g P m^{-2} d^{-1}) under 3-day HRT and 200-300 µmol·m^{-2}·s^{-1} light. Granular ABGS achieved ~75-85% TN and TP removal with ~1-2 day HRT. Biomass productivity ranged 10-30 g m^{-2} d^{-1} (HRAP) and 5-15 g m^{-2} d^{-1} (RAB) depending on light. Photosynthetic O_2 drove ~80-90% of nitrification demand, and CO_2 dosing at pH 7.8 enhanced growth. Sedimentable biomass (biofilm/granule) simplified harvesting. These results (summarized in Table 1) support ABC as an energy-saving, resource-recovering solution. We recommend using HRT ~3-5 d in HRAP or 1-2 d in RAB/ABGS, with pH ~8 CO_2 dosing. Further work should integrate anammox or other pathways for residual N, and explore seasonal control of photoperiod.

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