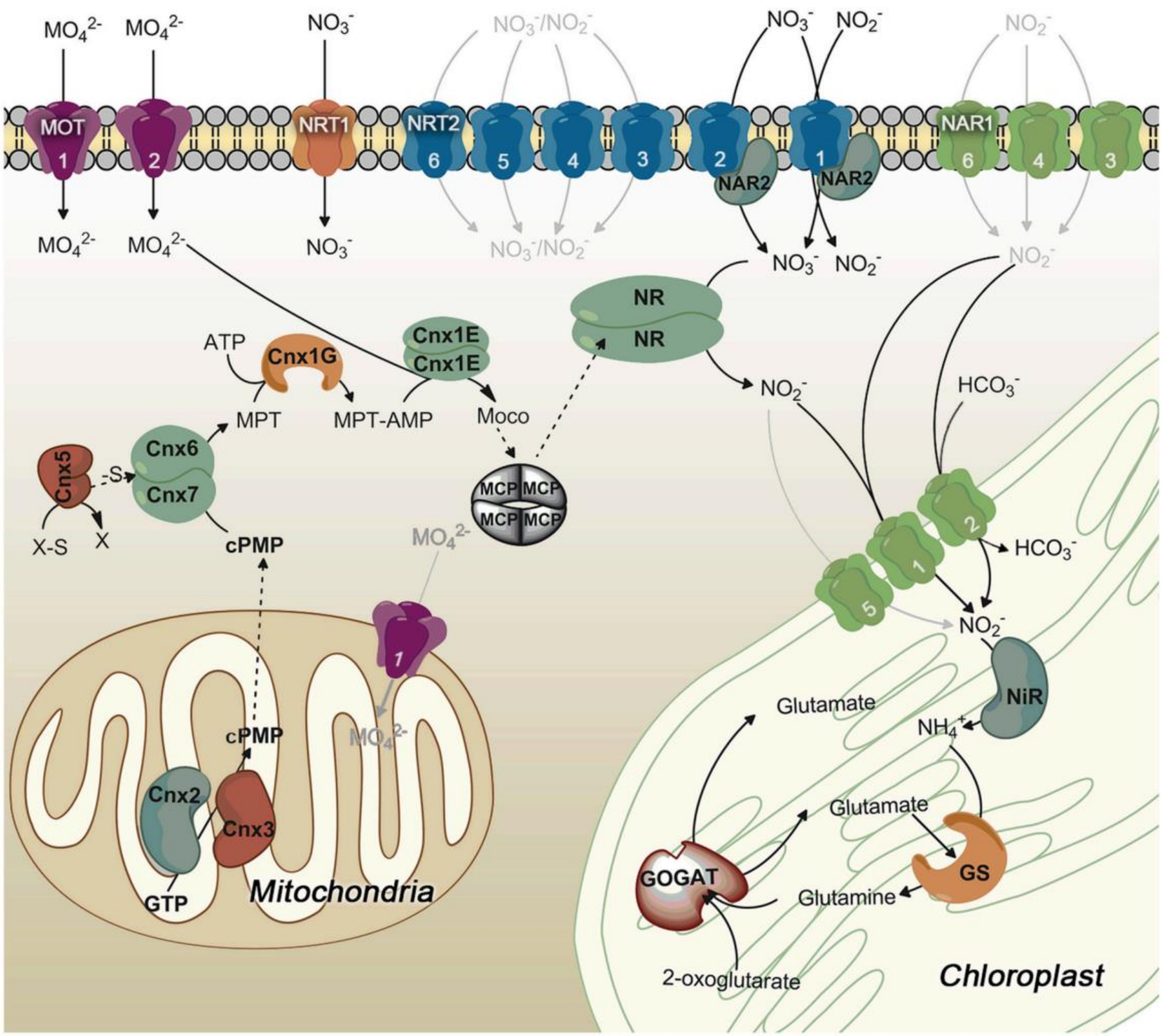
Nitrate Removal from Wastewater Treatments

Overall Pathway Overview:

Nitrogen assimilation is the process in which nitrates from the environment are converted into useable ammonium, which is then incorporated into organic nitrogen compounds for growth. This process is commonly used in all algae organisms to remove nitrates from wastewater.

General Sequence: External NO_3^- → Internal NO_3^- → NO_2^- → NH_4^+ → Organic N (Biomass)

Key Components and Mechanisms



Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A., & Fernandez, E. (2015). Understanding nitrate assimilation and its regulation in microalgae. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00899 (Sanz-Luque et al., 2015)

A. Nitrate and Nitrite Uptake

- Mechanism: Requires ATP (energy) for active transport to occur by moving nitrate and nitrites from a lower concentration to a higher concentration against concentration gradient.
- Key Transporters:
  NRT1 Transporters: They are from the NPF family. Although present in plants, their presence in the algal genome is generally low. They exhibit dual affinity (high affinity at low nitrate and low affinity at high nitrate) regulated by phosphorylation.
- NRT2 Transporters: From the NNP family. Present in the algal genome and highly relevant. Ability to transport both nitrates and nitrites through the membrane.
- NAR1 Transporters: From the FNT family. Found in model algae like *Chlamydomonas.*
  - NAR1.1: Specific for transporting nitrite (N02-) into the chloroplast
  - NAR1.2: Transports bicarbonate into the chloroplast and is a key component of the CO2 concentration mechanism, vital for efficient photosynthesis at low CO2

B. Nitrate Reduction (NO3- to NO2-)

- Enzyme: Nitrate Reductase (NR)
- Reaction: Catalyzes the reduction of Nitrates to Nitrites
- Location: Occurs in the cytosol
- Structure: A protein with subunits containing three prosthetic groups: FAD, b557 heme and Molybdenum Cofactor (Moco)
- Important Cofactor: Moco is highly essential for NR's main nitrate reduction. Moco biosynthesis is a complex pathway involving many proteins and occurs throughout the mitochondria and cytosol.
- Electron Donor: For the reduction to take place, NADPH provides electrons.

C. Nitrite Reduction (NO2- TO NH4+)

- Enzyme: Nitrite Reductase (NiR)
- Reaction: Catalyzes the reduction of nitrite to ammonium
- Location: Stroma of chloroplast
- Structure: Monomeric protein that consists of [4Fe-4s] grouping and siroheme prosthetic group.

- Electron Donor: Reduced ferredoxin provides electrons for this process to occur. It is generated from photosynthetic electron transport chain hence making this whole process highly light dependent

D. Ammonium Assimilation (Incorporation into Organic Nitrogen)
- Pathway: GS/ GOGAT Cycle
- Enzymes: **Glutamine Synthase** combines glutamate and ammonium to form glutamine. **Glutamate Synthase** uses glutamine and a carbon skeleton (2-oxoglutarate) to regenerate Glutamate
- Location: Occurs primarily in the chloroplast
- Importance: This is the final step where inorganic ammonium is converted to organic compounds (amino acids like glutamate and glutamine) which are then used as building blocks for making proteins and forming the final algal biomass.

Key Regulatory Factors

- **Ammonium (NH_4^+)** is a strong repressor of nitrate uptake. It can also reduce NR and NiR activity if there is a high presence of ammonium as a nitrogen source available within the cell.
- **Light** is essential for driving photosynthesis which provides:
  - Energy for active nitrate uptake (ATP, NADPH)
  - Reduces ferredoxin for NiR activity
  - Direct activation of NR
- Nitrate Availability: Induces the expression and activity of nitrate transporters and reductases.
- The availability of carbon skeletons (like 2-oxoglutarate) is critical for ammonium assimilation via the GS/GOGAT cycle, linking carbon and nitrogen metabolism.
- Signaling molecules like Nitric Oxide and cGMP can influence transcriptional and post-translational regulation of NR and transporters

Relevance to Chlorella Vulgaris Bioreactor Design for Water Purification

- Light optimization: Highly essential for optimal light intensity and light penetration for photosynthesis to occur and activate key enzymes
- Nitrogen Source Management: If the wastewater being treated contains both ammonium and nitrate, uptake of ammonium and its inhibitory effect on nitrate assimilation should be considered. This might require pre-treatment or a multiple stage process to avoid high ammonium levels

- Molybdenum Supplementation: Ensuring the right amount of Molybdenum present is critical as it is a non-negotiable cofactor for NR.
- Carbon dioxide supply: The link between nitrate assimilation and carbon metabolism means a correct amount of CO_2 should be present to remove nitrogen and promoting algal growth
- Hydraulic Retention Time: The bioreactor must provide sufficient time for all of the enzymatic processes to occur effectively
- Biomass Harvesting: Since nitrate is transformed to algal biomass, it is important to efficiently harvest the grown algae physically. This is the ultimate step to physically remove nitrogen from treated water.

Growth of Chlorella Vulgaris in high concentrations of Nitrate and Nitrites for Wastewater treatment

1. Core Identity & Big Picture (5-10 minutes)

- **Main Objective/Research Question:** The study looks at the effects of high nitrate and nitrite concentrations on cell growth and nitrogen removal of Chlorella Vulgaris.

- **Key Organism/System:** This study demonstrated the potential of Chlorella Vulgaris to efficiently remove and utilize both nitrates and nitrites from a culture medium even at high initial concentrations while achieving high biomass productivity."

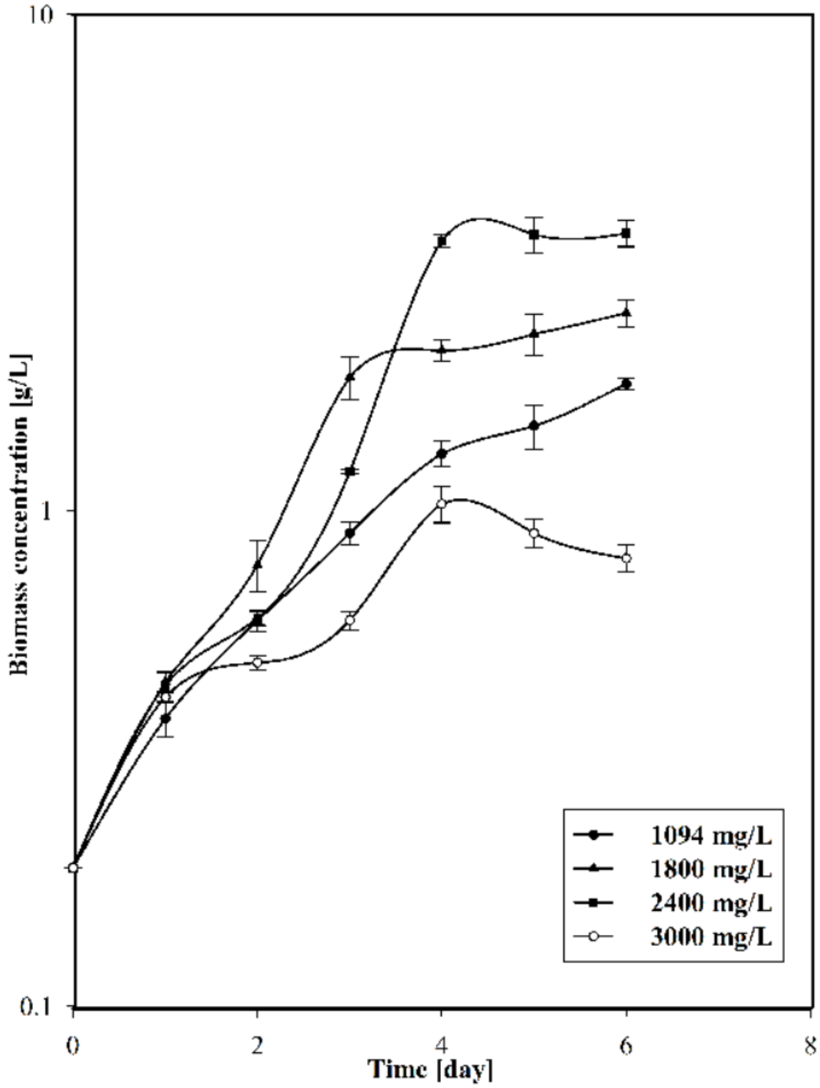
- **Headline Finding(s):** Chlorella vulgaris is exceptionally robust, capable of efficiently removing and utilizing high concentrations of both nitrate and nitrite, while simultaneously achieving high biomass productivity, making it an excellent candidate for treating nitrogen-rich wastewater.

2. Methodology

- **Experimental Setup Type:** The algae were injected using log phase cells to a 250mL of culture media in cotton-stoppered 500mL Erlenmeyer flasks
- **Key Culture Conditions:**
    - **Temperature:** 27±1°C
    - **Light:** 4000 Lux with a 12:12 hour light dark cycle
    - **Medium:** BG-11 medium was used
    - **PH:** was set to 7 before autoclaving
    - **Inoculation:** Culture were started with a 5% Vol:Vol log phase cells
    - **Aeration/Mixing:** Flasks were incubated on a rotary shaker at 100rpm and aerated at 1vvm with filtered air
- **Main Variables Tested:** The study tested different initial concentrations of nitrates and nitrites in the culture media
- **Key Measurements:** The researchers primarily measured **algal biomass** (determined by dry cell weight and optical density at 680 nm) and the daily **concentrations of nitrate and nitrite** in the culture supernatant.
- **Duration of Experiment:** 8 to 10 days long

3. Detailed Results & Data Extraction

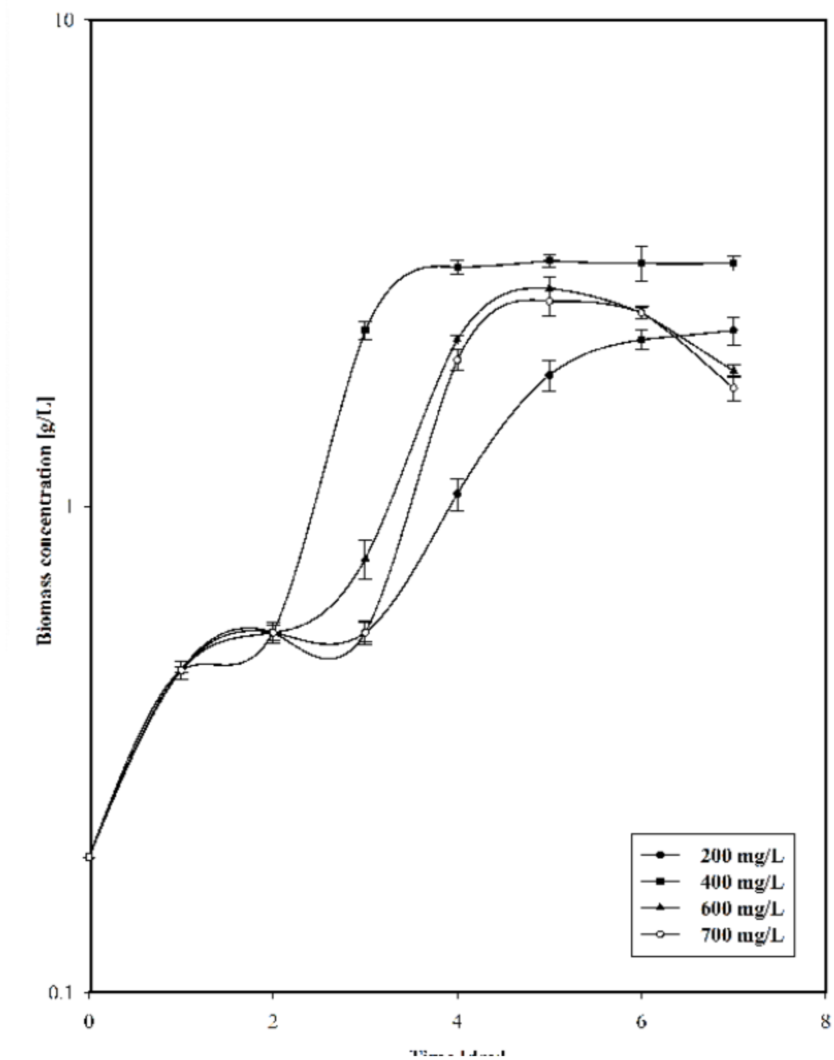
- **Figure 1:**



- **What does the figure show:** The growth rate and biomass accumulation was low at initial nitrate concentrations of 1094mg/L. The maximum biomass concentration was reached at 2400mg/L. When the concentration of nitrate increased to 3000mg/L, the biomass concentration dropped significantly. This suggests that an optimum initial nitrate concentration is required to produce efficient biomass production.
- **Key Data:**
  - The fastest growing curve is at 2400mg/L nitrate peaking at around day 4.
  - Growth at 1800mg/L is also shown to be strong but slightly lower than 2400mg/L.
  - Growth at highest concentration, 3000mg/L, is significantly less compared to 1800 and 2400mg/L
  - Most cultures reached stationary after day 3-4

- **Relevance to Bioreactor Design:**
  This graph shows that optimal nitrate concentration between 1800 – 2400 mg/L is required for maximizing Chlorella Vulgaris biomass production. An increase in nitrate concentration (3000mg/L) is the tolerance limit, suggesting an increase above this concentration can cause reduced performance. The rapid growth and stationary point at day 3 –4 is a direct input to determining Hydraulic Retention Time (HRT).

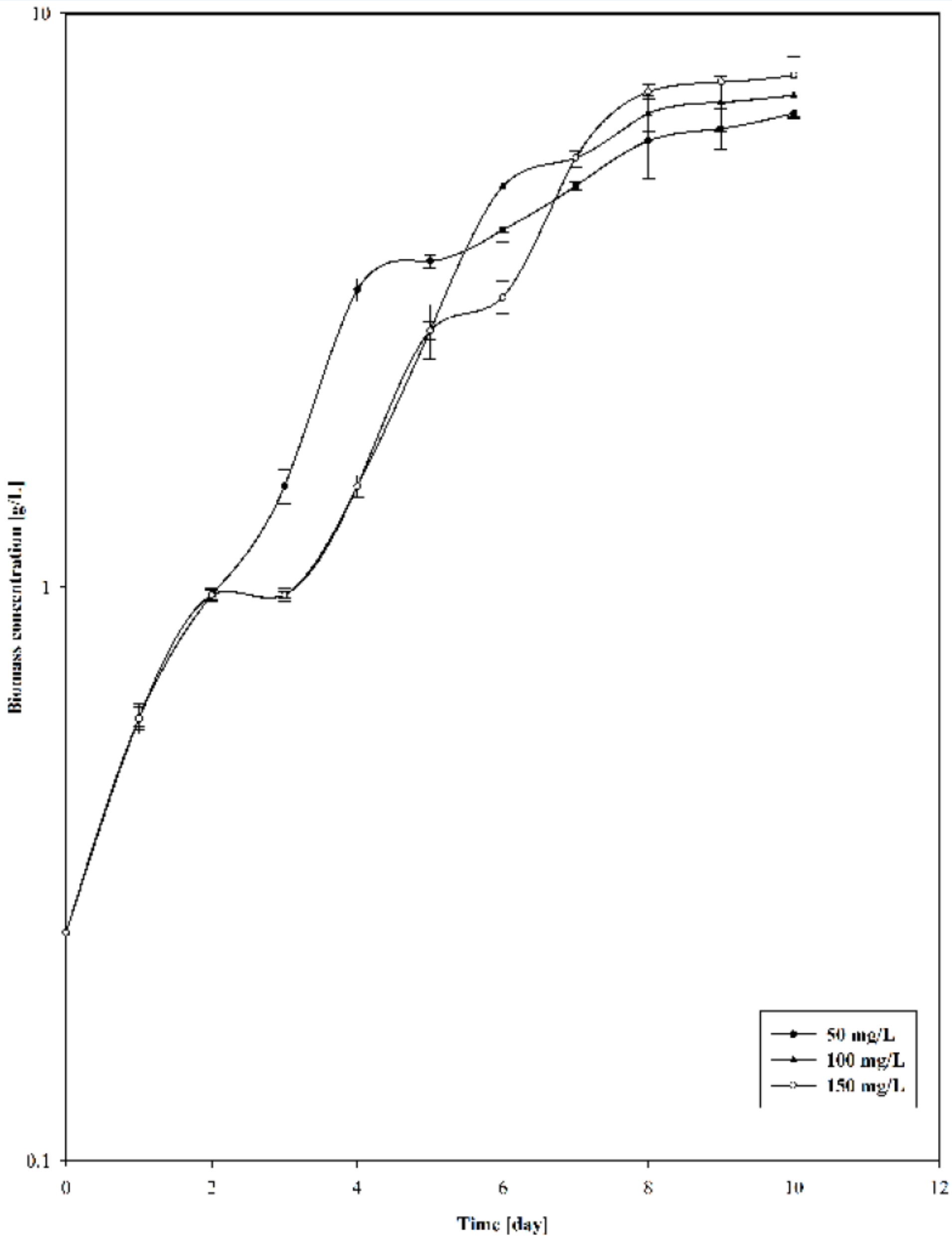
- Figure 2:



- **What the graph shows:** This graph shows the growth kinetics of Chlorella vulgaris with nitrite as the sole Nitrogen source.
- **Key Data**:
  - Initially all four concentrations increased and then reached a lag phase of 1-2 days.
  - 400mg/L showed the fastest growth and highest biomass concentration and reached a plateau by the 4th day.

- **Relevance to Bioreactor Design:**
  The graph helps pinpoint that 400mg/L is an optimal concentration of nitrite to produce maximum amounts of biomass using Chlorella Vulgaris. In addition, the lag phase for the reaction to start is about 1-2 days. To reach a plateau and constant biomass requires 5-6 days to complete the process. This suggests the hydraulic Retention time should be around 6 to 7 days.

- Figure 3:



  - **What does the graph show?** This graph shows the biomass production when nitrate is present, and supplemented with additional nitrite concentrations of 50, 100 and 150mg/L. It shows an efficient increase in biomass productivity with an increase in time.

- **Key Data:**
  - All cultures showed a robust growth reaching higher biomass concentration (7-8g/L for 150 mg/L nitrite) compared to cultures grown with only nitrate or nitrite conditions.
  - Cultures supplemented with 100mg/L and 150mg/L nitrite exhibit a one-day lag phase between day 2 and day 4. In contrast, the culture with 50mg/L nitrite does not show this lag.
  - After the lag, the cultures in 100 and 150mg/L nitrite resume logarithmic growth, reaching higher final biomass than 50mg/L nitrite culture.
- **Relevance to Bioreactor Design:**
  This graph is pivotal as it suggests that combining nitrate and nitrite leads to a substantially higher *Chlorella vulgaris* biomass yield, making it highly relevant to the bioreactor design for this project. Therefore, it is crucial to consider the Hydraulic Retention Time (HRT), especially when managing mixed nitrogen loads, because a transient growth lag at higher nitrite supplementations is a critical factor.