DENITRIFICATION OF WASTEWATER USING CYANOBACTERIAL-ALGAL MATS WITH SODIUM BICARBONATE

A Major Qualifying Project
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WORCESTER POLYTECHNIC INSTITUTE
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in
Environmental Engineering

by
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Report Submitted to:

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Abstract

This project examined the efficacy of solid sodium bicarbonate as a low-cost, viable substitute for carbon dioxide gas in small-scale cyanobacterial-algal (microbial) mat agricultural wastewater treatment. Experimentation over 35 days with nitrate-rich wastewater resulted in removal efficiency rates up to 37.2% of NO_3 for microbial mats treated with solid sodium bicarbonate. Reaction rate modeling determined a rate constant of 0.0166/day and Excel modeling determined a projected hydraulic residence time with increased microbial mat area for an estimated 75% removal. Recommendations include further long-term experimentation and suggestions for an initial prototype design.

Acknowledgements

I would like to extend deep gratitude to the staff of the Arava Institute of Environmental Studies (AIES) on Kibbutz Ketura in Israel for sponsoring and hosting my Major Qualifying Project (MQP) work abroad. Dr. Gabi Banet, an educator and researcher with AIES and the Dead Sea & Arava Science Center, was instrumental in the design and implementation of the experimental portion of this MQP, and without him this project wouldn't exist. I would also like to thank Isa Bar-On of the Mechanical Engineering department at WPI for her structural and organizational guidance on-site, and John Bergendahl of the WPI Civil and Environmental Engineering department for his crucial technical assistance while I was abroad, and his continued guidance on data analysis upon returning to Worcester. Finally, I would like to thank all of the AIES and WPI peers/colleagues that supported my work and my family for helping me reach my goals.

Professional Licensure

Earning a Professional Engineering license (PE) is crucial for higher level engineering professionals that wish to design, approve, and implement a functional system. A PE license ensures the engineer has been awarded a degree in engineering from an ABET accredited school, trained as a junior level engineer, and mastered the theory of their field, according to the National Council of Examinations for Engineering and Surveying (NCEES). Before earning a PE, one must pass the Fundamentals of Engineering exam (FE) to receive an Engineer-In-Training (EIT) license. The FE exam is a 6-hour test given in two sessions.

Eligibility based on number of training hours varies by state, for EITs looking to take the PE exam. A license can be revoked if an engineer does not abide by the code of ethics, and he/she may be held fiscally and professionally responsible if a design fails to meet set criteria. The purpose of a PE license is to protect the public and hold engineers accountable for their work. The current design of a pilot batch bioreactor prototype for wastewater denitrification is preliminary and would require final approval by a PE, if implemented.

Design Statement

The Accreditation Board of Engineering and Technology (ABET) criteria for capstone design is as follows:

"Students must be prepared for engineering practice through the curriculum culminating in a major design experience based on the knowledge and skills acquired in earlier course work and incorporating engineering standards and realistic constraints that include most of the following consideration: economic; environmental; sustainability; manufacturability; ethical; health and safety; social; and political."

The design process for this project was conducted in two separate portions: the on-site experiment, and the data analysis and prototype modeling. Socio-economics, sustainability, and ethics were heavily factored in the design process.

The experiment was designed using data obtained from previous research pertaining to microbial mat denitrification processes. A control condition was developed using CO_2 feeds and lab-grown microbial mats. A standard surface area of mat was selected, and six identical tanks were setup. The three experimental tanks required calculations to determine appropriate amounts of sodium bicarbonate to add. Proper laboratory procedure and scientific methods were followed.

The prototype design required reaction rate modeling using first order kinetics and graphical modeling and data fitting in excel. The design for the pilot batch bioreactor required the use of UV-resistant and weak-acid tolerant materials, to prevent damage due to precipitate buildup and maintenance. Hard plastics are a low-cost material and easily accessible in many areas. Pump size was adjusted for increased Q, hydraulic residence time (HRT) is theoretical until further experimentation is conducted. Suggestions for three subsequent experimental processes that would increase accuracy of reaction modeling were outlined.

Executive Summary

The goal of this MQP was to develop a potential solution for desert-region agricultural wastewater recycling. Water high in nitrates cannot be readily reused for agricultural application if too heavily loaded, as excess nitrate (> 40 ppm) causes depletion of other essential nutrients in the plant and soil and, eventually, renders soil infertile. Lysimeter wastewater from the Southern Arava R&D Experimental Farm is currently dumped to septic for groundwater recharge. The sample removed from the lysimeter and used in the laboratory contained an average of 580 mg/L (ppm) NO_3^-, well outside acceptable re-use range.

Nitrogen uptake is one beneficial function of cyanobacterial-algal (microbial) mats, filamentous auto- and heterotrophic organisms grown on a mesh screen. Similar to fixed film reactors, microbial mats effectively treat nitrogen species in wastewaters, with previous experimentation resulting in 80% total N reduction in aquaculture effluent. However, these systems require the use of carbon dioxide gas as a carbon source for oxidation of nitrates, which can be cost prohibitive and unavailable in more isolated regions.

This project examined the efficacy of solid sodium bicarbonate as a low-cost, viable substitute for carbon dioxide gas in small-scale microbial mat agricultural wastewater treatment. Experimentation over 35 days with nitrate-rich wastewater resulted in removal efficiency rates up to 37.2% of NO_3^- for microbial mats treated with solid sodium bicarbonate. Figure 1, below, shows the averaged nitrate reductions for sodium bicarbonate experimental tanks and carbon dioxide control tanks.

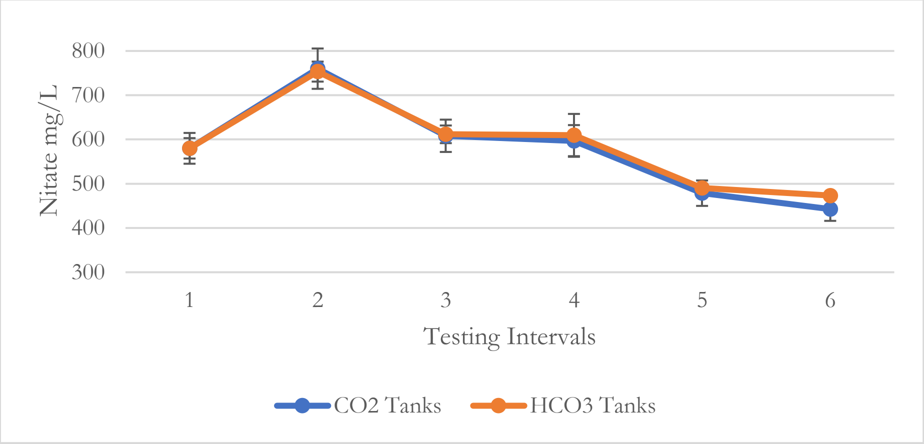


Figure 1. Average Nitrate Concentrations from t0 to t35 for experimental and control microbial mat tanks.

Reaction modeling determined a k value of 0.0166 day^{-1} and loading rates of 5.4 mg NO_3^- per cm^2 of mat. Figure 2, below, shows experimental data and theoretical removal rates. Modeling suggests increasing microbial mat area for more efficient removal. Recommendations include further long-term experimentation and suggestions for an initial prototype design.

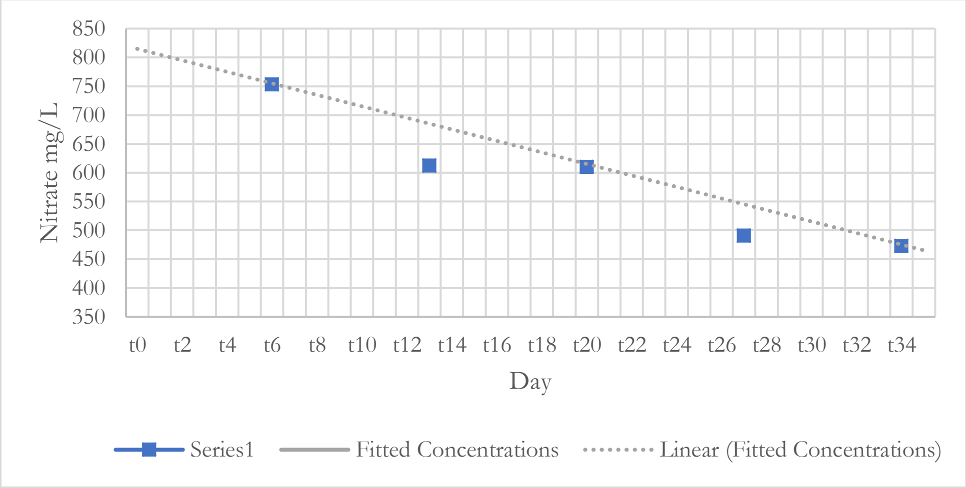


Figure 2. Reaction rate concentrations fitted to sodium bicarbonate experimental data.

Contents

List of Figures

1 Introduction

1.1 The State of Israel

Israel is a country in the Middle East bordered by the Mediterranean Sea to the west, Jordan to the east, Egypt in the southwest, Syria and Lebanon in the north, and the Red Sea to the south.

The State of Israel is experiencing an economic boom in both incoming population and tourism rates. As a result, housing, transportation, and agricultural infrastructures are expected to expand to meet these growing demands. Israel has a current estimated population of 8.97 million and a population density of 407/km^2.[1] In October 2018, the country welcomed 486,000 tourists to the state, marking the highest rate of entry for a single month.[2] The Center Bureau of Statistics determined that an excess of 4 million tourists visited the country in 2018.

Figure 1: Map of the State of Israel

1.1.2 The Negev Region of Israel

As seen in Figure 1, 60% of the country is comprised of the Negev Region, a deserted and mountainous stretch that expands from the middle of Israel to the southern border and the Red Sea.[3] The southernmost city in Israel is Eilat, a major port and resort locale at the tip of the Arava Valley. The Arava Valley is home to eleven kibbutzim, egalitarian, self-sufficient communities that serve as the "towns" of the region and the "suburban" area stretching north from Eilat.

1.1.3 The City of Eilat

The city of Eilat is a major domestic tourism hub in Israel with a year-round resident population of 50,724,[1] boasting beautiful geological formations, warm waters with abundant coral communities, and seaside resorts. Mirroring trends across the entire country, Eilat is experiencing an increase in international tourism and immigration. The city developed blueprints last year to construct over 18,000 new permanent housing units to prepare for a spike in residential growth.[4]

1.1.4 Kibbutzim

There are 11 kibbutzim in the Southern Arava Valley: Eilot, Elifaz, Be'er Ora, Samar, Yotvata, Grofit, Ketura, Lotan, Neve Harif, Neot Smadar, and Yahel. These communities, mapped in Figure 2, range in size, prosperity, and social structure. Each kibbutz is geographically and infrastructurally distinct from the next, barring energy grid connection.

Each kibbutz has a unique treatment system for wastewater; Kibbutz Lotan and Neot Smadar have artificial wetlands that seek to serve as filtration and nutrient uptake mechanisms.

They are self-governing and self-deterministic, with representatives from each kibbutz serving on the Eilot Regional Council. The focus of the cooperative efforts of the Council are renewable energy projects and agricultural research.

Figure 2: Map of Kibbutzim in the Arava Valley.

1.1.4.1 The Arava Institute for Environmental Studies (AIES)

Located on Kibbutz Ketura is the AIES, a multi-national collaborative education and research program focusing on environmental concerns in the region. The program houses students for two semester sessions, and educators conduct classes on environmental policy, stewardship, and ecology. The ultimate goal of AIES is to foster "beyond borders" mentalities when it comes to cooperative policy-making in the Middle East. Dr. Gabi Banet is a researcher and educator with the

AIES and the Dead Sea & Arava Science Center. His previous research related to this project focused on denitrification of aquaculture effluent (section 2.8.4.1).

1.1.4.2 Southern Arava R&D at Kibbutz Yotvata

Kibbutz Yotvata is situated in the Arava Valley region within the Negev. One of the largest kibbutzim in the area, it is home to the regional school and clinic, a large dairy farm, and agricultural research facilities (Southern Arava R&D). The foci of the experiments on the R&D farm are salinity and water use: how to get the largest crop yield with the least amount of freshwater. The researchers are partnered with other R&D farms across the State.

1.2 Soil Composition in the Arava Valley

The Negev sees little rainfall, with the winter months characterized as the “rainy” season, during which pressure systems form rapidly across the mountain ranges and precipitation falls in large volumes for short storm periods. Due to the arid soil, water does not readily penetrate the top soil and flash floods have been known to occur as a result of runoff.[13]

The soil is composed of sand and clay compositions, with slow transport of groundwater through the sediment. New regulations in the region have phased out septic and cesspool wastewater dumping methods to protect well and aquifer water quality. [13, 11]

1.3 Groundwater and Aquifer Recharge

Freshwater is extremely scarce in this region; less than 1% of potable water worldwide is stored in the Middle East and North Africa, with 87% of that water used for agricultural applications.[6]

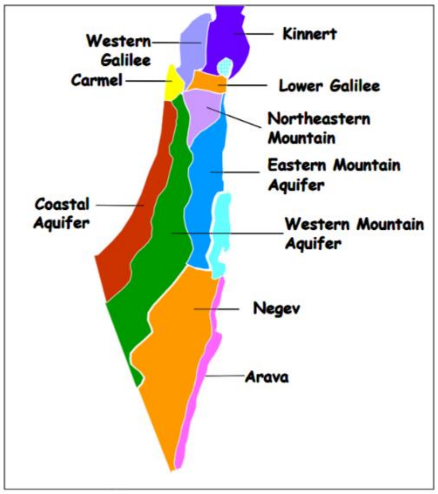


Figure 3: Denotation of Hydrological Unit Boundaries

There are three hydrological units of aquifer groups located in the Negev and Arava valley: the Hazeva and Dead Sea, the Judea, and the Kurnub. The Hazeva and Dead Sea groups are confined within sedimentary rocks, clastic alluvium, and varying clays. The Judea group is mainly composed of hard carbonate geological deposits of limestone and dolomite, while the Kurnub group is comprised of sandstones, silts, clays, and some shale deposits.[11]

The estimated rate of recharge from rainfall for all three groups is 32 mcm/year, however 28 mcm/year of that water is highly saline, containing chloride concentrations above 400 mg/L. Additional sources of aquifer recharge include water released from fossil storage and pressurized deep brine reservoirs.[11]

1.4 Water Usage in the Negev

1.4.1 Israeli Desert Agriculture

In the 1930s, Simcha Blass invented drip irrigation, a technique that pipes water directly and systematically to the root systems in need. By using less water than conventional methods, drip irrigation enabled large-scale desert agriculture to succeed in the Negev and is still in practice today.[13]

The Southern Arava R&D farm has modified drip irrigation practices to serve for large crops of coriander, basil, pomegranate trees, and other flowering, agricultural, and herbaceous plants. Many of the experimental crops make use of lysimeters: collection basins beneath the root system of the plant that gather and weigh the unutilized water to measure percent uptake. Researchers vary nutrient content and salinity to identify different conditions that might affect water uptake for a plant root system. The water collected in lysimeters is then piped to septic or cesspool for groundwater recharge which, as seen in section 1.2, is being phased out in the region.[14]

One major source of excess lysimeter effluent are date trees. Known for being tolerant of high salinity, excess nutrient, and low water quality, they are commonly integrated into experiments on the farm. The date field produces 300-400 liters/day of effluent wastewater high in nitrates, salinity, and dissolved ionic content that is piped directly into a cesspool.[21]

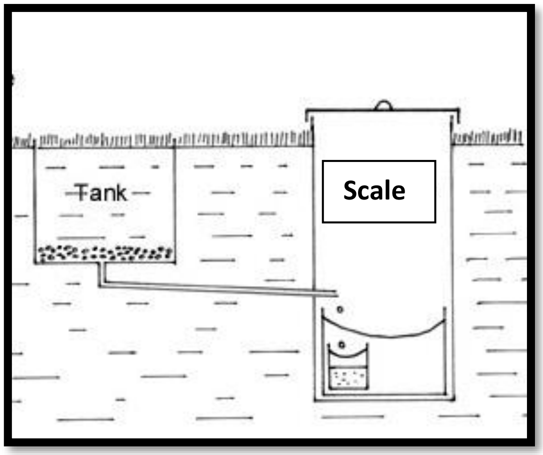


Figure 4. Basic layout of lysimeter system.

2 Background

2.1 Agricultural Wastewater from Southern Arava R&D

The farm is home to a small-scale desalination plant, which treats water pumped from the Kurnab, Judea, and the Hazeva and Dead Sea Group Aquifers: limestone- and sandstone-confined groundwater collection points for the Negev and Arava basin. 89% of the groundwater that recharges these aquifer groups annually contains chloride concentrations over 400 mg/L.[11] This means that the majority of the aquifer is recharged with water that is highly saline: >200 ppm / 312.5 EC/ 0.3125 (dS/m). Additionally, groundwater from ocean bodies and highly-pressurized brine reservoirs underground seep into aquifers and increase salinity.[8]

2.2 Nitrate contamination

Nitrate contamination is dangerous in aquifers and wells that are to be used for consumption. It can cause what is colloquially known as “Blue Baby Syndrome”, effectively starving red blood cells of oxygen, eventually deeply injuring or killing a small child. Currently, the EPA standard maximum contaminant levels (MCL) for nitrate is 10 mg/L.[22]

2.3 Average Hours of Sunlight per Day in Israel

The average maximum hours of daylight in the summer is 14 hours and 3 minutes. In December and January, that average dips to 10 hours and 15 minutes. This experiment was conducted under winter conditions, which provided diminished availability of photosynthesis to autotrophic organisms. Therefore, results of this experiment indicate the lowest bioproductivity rates in microbial metabolic range using ambient light.

2.4 Redfield Ratio

The Redfield Ratio, first described by A.C. Redfield, an oceanographer in the 1930s-1950s, identified that marine auto- and heterotrophic planktons and algae have a set ratio of dissolved nutrients to particulate matter as it stoichiometrically relates to total Carbon, Nitrogen, and Phosphorous concentrations. The Redfield Ratio is often expressed as C:N:P → 106:16:1.[23] While there are variations in ratios dependent on the particular ecosystem, 106:16:1 is valid for of estimating the stoichiometric assimilation of nitrogen in carbon based, aquatic systems.

2.5 Denitrification

The process of reducing nitrate to nitrogen gas is called denitrification. Nitrate is unsafe to have present in drinking water, often regulated by MCL (section 2.2) and must be removed to satisfy set levels in both traditional and alternative wastewater treatment methods if the effluent is intending to recharge aquifers through groundwater. The chemical reaction of denitrification is provided below.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

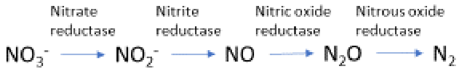


Figure 5. The process of denitrification

2.6 Deionized water

Well-pumped and other natural sources of water often contain dissolved mineral ions which can affect chemical reactions and pH levels when used in experimental applications. Deionizing water for experimental use reduces ionic content and ensures minimal noise in data when measuring nitrogen compounds.

2.7 Carbonate Uptake

Reduction of nitrate and nitrite requires an available biodegradable carbon source. According to Droste, any carbon species can degrade nitrates to varying efficiencies.[19] The following half-reactions detail the process of denitrification of NO_3^- via HCO_3^-:

$$2NO_3^- + 12H^+ + e^- = N_2(g) + 6H_2$$

$$H^+ + \mathrm{HC}O_3^- = CO_2 + H_2\mathrm{O}$$

$$2NO_3^- + 13H^+ + \mathrm{HC}O_3^- \rightarrow N_2(g) + CO_2 + 7H_2O$$

From this oxidation reaction, total bicarbonate consumed can be calculated using the molecular weights of all species.

2.8 Microbial Mats

Microbial mats are filamentous structures of microalgae, cyanobacteria, and other hetero- and autotrophic organisms that use CO_2 and UV energy to produce biomass. Frequently found in aquatic ecosystems, they also can inhabit deserted regions, highly saline environments, and both below freezing and above boiling temperatures.

2.8.1 Nitrogen Uptake

Microorganisms present on the mat are capable of assimilating nitrates into nitrites, nitrites into nitric oxide, and finally reduces to gaseous N_2, which diffuses to the atmosphere. [19]

$$NO_3^- + 8H^+ + 8e^- \rightarrow NH_3 + 2H_2O + OH^-$$

2.8.2 Source of Microbial Mats

The microbial mats used in this experiment were grown in the lab at Ma'aleh Shacharut on Yotvata. The following data was provided by Dr. Gabi Banet from his mentorship for a Master's thesis series of experimentations utilizing microbial mat wastewater treatment for fish effluents. The wastewater contained 20 mg/L NO_3^-. The majority of micro-organisms growing on the mat were two cyanobacteria species, Leptolyngbya and Spirulina, and Liagora and Paulinella algae species, which can be seen in Figures 7 and 8, in section 2.8.4.1.[15]

2.8.3 Fe^{3+} to enhance mat productivity

Previous experimentation with microbial mat bioproductivity was conducted under the guidance of Dr. Gabi Banet. Julia Kabasnicki, a graduate researcher with the Dead Sea & Arava Science Center, conducted experiments that determined microbial mats treated with both CO_2 and an Fe^+ solution in the water would increase mat growth and nitrogen removal capabilities.[16]

2.8.4 Potential Alternative Wastewater Treatment Method

Certain autotrophic organisms are capable of assimilating nitrogen and thus have the potential to be used as a treatment for agricultural wastewaters high in Total Nitrogen.[17, 10] This renders complex symbiotic, filamentous microbial mats capable of both nitrification and denitrification, depending on external environmental factors/nutrient sources.

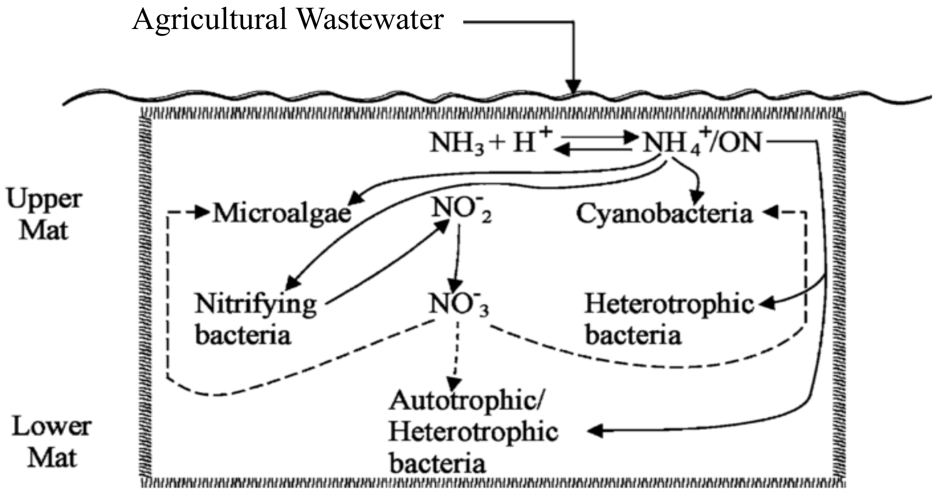


Figure 6. Microbial mat nutrient uptake diagram[16]

2.8.4.1 Previous Experimentation with Microbial Mats and Wastewater

- A literature and research review identified experiments over the past 50 years that have focused on microalgae and cyanobacteria wastewater treatment capabilities. The authors found that much of the data supports the theory that microbial mats are not only capable of Total Nitrogen removal, but also heavy metal removal, phosphate removal, and can be inhibitive of certain types of coliform growth. [6]

- Guangmin et al. researched bicarbonate capture of two microalgae species: *Chlorella* and *Scenedesmus obliquus*s. Using a continuous flow bioreactor and analyzing NH_4^+-N removal via HCO_3^- uptake, they found that the microalgae used 63.9% of available bicarbonate to reduce total NH_4^+-N by 80%.[7]
- Dr Gabi Banet advised Svet Verhovskiy's series of experiments related to denitrification capabilities of microbial mats with aquaculture effluent. Genetic analysis of the lab grown mats determined the relative abundance of the most common cyanobacteria and algae species present, seen in Figures 7 and 8.[15]

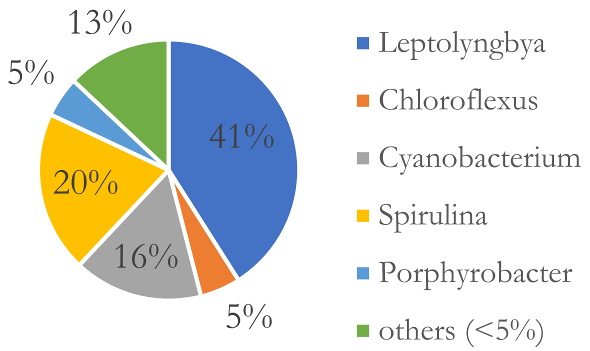


Figure 7. Cyanobacteria initial relative abundance by species

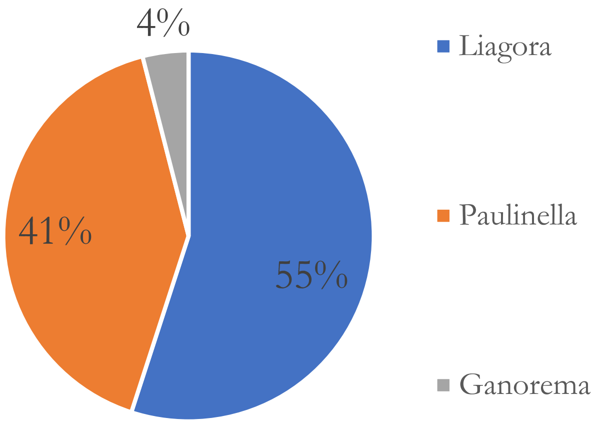


Figure 8. Algae initial relative abundance by species

Verhovskiy's experiments found an 81% efficiency in Total Nitrogen and NO_3^- removal from fish effluent, showing the capabilities of microbial mats as a potential in-situ treatment for agricultural wastewater. Ideal operating temperature between 30°-40° C, with 3 g/L added salts resulted in 1.6 g TN/m^2d removal.[15]

With autotrophs that are O_2 producing, a feed of CO_2 is necessary for functional biomass development as a means to assimilate nitrogen species. Currently, the cost of CO_2 tanks, feeder lines, and pressurized controls represents at least 50% of the overall cost of microbial mat treatment.[14] This can be a prohibitive cost in many situations, and too complicated to install for a variety of desert agricultural wastewater recycling systems.

2.9 Project Sponsor and Goals

The goal of this project, as directed by AIES, Southern Arava R&D, and under the guidance of Dr. Gabi Banet at the Ma'aleh Shacharut Regional School, is experimentation towards the design of an in-situ agricultural wastewater recycling treatment for potential application at the R&D farming station, and, more broadly, any small-scale desert climate application. Experimental wastewaters rich in nitrates are collected in lysimeters and dumped to septic to recharge the groundwater, furthering contamination of the surrounding soils and aquifer. Additionally, water is a precious resource in the desert, and innovative wastewater recycling design has a prominent role in the progress of the region. A potential solution to this issue was examined throughout experimentation and design work.

2.9.1 Hypothesis

The expectation of the experiment is that HCO_3^- , introduced in the form of $NaHCO_3$, will serve as an alternative source of CO_2 for microbial mat growth and subsequent total nitrogen removal from agricultural wastewater.

3 Methodology

3.1 Bench-scale experimentation with bicarbonate

The following materials were required to operate the experiment and to perform necessary analysis:

- Six 30 cm x 15 cm x 16 cm glass aquarium tanks
- Prefabricated plastic and aluminum foil sleeves and lids to prevent photosynthetic organisms from growing inside the tanks
- 6 Atman AT 101 water pumps, max Q=500 L/hr
- 6 Atman AT-50W water heaters
- Six 12 cm x 9 cm x 6 cm Tupperware converted to fit effluent, tubing with nozzles, and influent CO_2
- Plastic tubing and irrigation nozzles
- Storage tank of CO_2, Advanced Specialty Gas Equipment distribution system
- CO_2 distribution lines for control tanks
- Solid sodium bicarbonate _ % pure, from _manufacturer
- Deionized Water from a Zalion Conductivity Meter
- Fe^{3+} ion solution
- Merck RQflex
- Thermometer
- Merck Nitrate test trips (5-225 mg/L)
- Merck Nitrite test strips (0.5-25 mg/L)
- Vacuum pump
- Advantec glass fiber filters – 47 mm (GC-50)
- Microwave Oven
- Witt Oxybaby M+ CO_2 meter
- Adwa AD1030 pH/mV meter
- hot glue gun for repairs to system
- Six 7cm x 9cm sections of microbial mat, dominant sp. Leptolyngbya

The tanks were set up in an available lab within the greenhouse, using only ambient winter sunlight conditions. Each tank received a pump, a water heater, and 4 liters of untreated wastewater. The height of 4 liters in each tank was 9.5 cm, which indicated where to replenish evaporated wastewater with deionized water when necessary to maintain constant volume. Labeled right to left, the tanks are # 1, 2, 3, 4, 5, and 6. Tanks 1-3 represent the control conditions, as previous experimentation with CO_2 has shown successful nitrogen species removal.

The influent water line to the Tupperware was securely inserted into the pump and routinely checked for disconnection.

The heaters were all set to 35 C+, and temperature was periodically recorded to ensure a range of 35-40 C.

100 µL of Fe^{3+} solution was added to each tank to aid in microbial mat growth, determined to be best standard practice by Dr. Gabi Banet for microbial mat wastewater treatment systems.

Into the 3 tanks to the right, CO_2 lines were placed into a hole cut in the Tupperware. This fed a constant stream of 2.1-2.2% CO_2 air into the microbial mat headspace.

The 3 left-most tanks were not fed CO_2 air, but were treated with sodium bicarbonate, as detailed below.

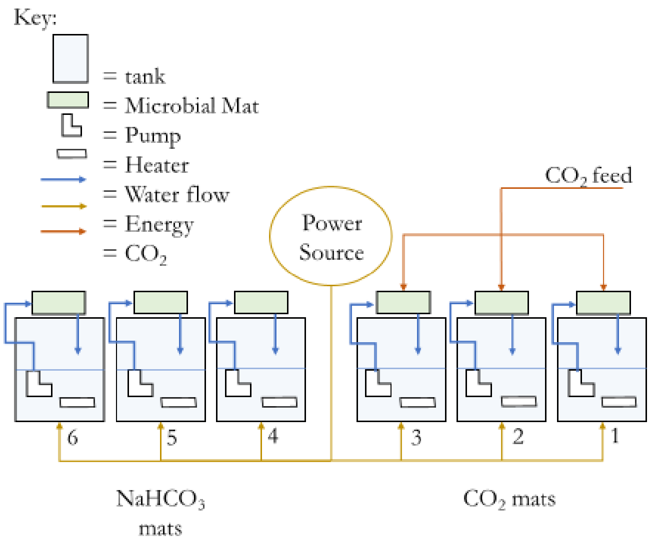


Figure 9. Front view of experimental

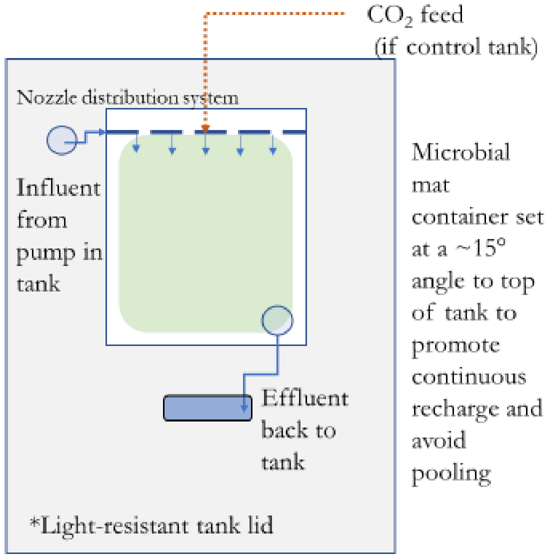


Figure 10. Plan view of individual mat and tank setup experimental layout.

3.1.1 Calculation of Sodium Bicarbonate Weight

Prior to testing, the agricultural wastewater was estimated to have a ~100 ppm nitrate content. Initial calculations, as follows, determined that 4.3 g of solid sodium bicarbonate would provide enough carbon dioxide to solution to denitrify 4 liters of 100ppm nitrate wastewater:

$$\left(\frac{100\ mg\ NO_3}{\frac{62g\ NO_3}{mol}}\right) * \left(\frac{14\ g\ N}{mol}\right) = \frac{23\ mg\ N}{L}$$

$$\frac{23mgN}{L} * 4L = 92\ mg\ N$$

The goal then was to assimilate 92 mg of total N. Using the Redfield Ratio (section 2.4), the total weight of $NaHCO_3$ required was calculated. Sodium bicarbonate was introduced twice, the first dose calculated by dividing the total weight by 2.

$$(92\ mg\ N) * \frac{106}{16} = 298\ mg \rightarrow \frac{298\ mg\ C}{\frac{12g\ C}{mol}} * \frac{84gHCO_3}{mol} = 4.3\ g\ NaHCO_3$$

~2.15 g of sodium bicarbonate was added to experimental tanks # 4, 5, and 6.

However, upon receival of the sample, testing with the Merck RQflex kit determined values of 580 ppm. The calculation of initial ppm was done in a series of dilutions 10-fold (1mL wastewater in 9 mL DI), 5-fold (1ml wastewater in 4 mL DI), 4-fold, and 3-fold. The original sample was kept in a sealed, light-resistant container. Interviews with Southern Arava R&D researcher Ehud Zalim revealed that historical nitrate ppm in date tree lysimeters was around 500 ppm and had been underestimated to be 100 ppm (Appendix C)

The high value resulting from the 10-fold value was considered an outlier, thus the three lowest dilutions were averaged to estimate t0 nitrate content. The average of the dilution scales and associated nitrate content resulted in a ~580 ppm value, which was used as the estimated t0 nitrate content of the wastewater.

An additional 4.1 g of $NaHCO_3$ was added directly to tanks #4, 5, and 6 and stirred. $NaHCO_3$ has a solubility of 96 g/L at 20 C. Solid white precipitate formed upon addition of sodium bicarbonate.

Due to precipitate formation in the $NaHCO_3$ tanks, a solution of 100mL DI water and 5.0 g $NaHCO_3$ was made and added directly to the microbial mats in lieu of solid $NaHCO_3$. This reduced precipitation rates. Samples of the original wastewater were sent to an outside laboratory in Eilat for in-depth water content analysis. Details of process and conclusions in Results section.

3.1.2 Microbial Mat Source and Biomass Calculation

The sections of microbial mat were cut from the same mat grown in the lab by Dr. Gabi Banet. They were fed with BG11 substrate and had grown for approximately one month before applied to the experimental system. The mat was grown on metal mesh screen. Initial dry weight (DW) biomass was recorded by cutting sections of original mat, drying via microwave oven, and weighing via analytical balance.

- Weigh single glass filter on analytical balance, record, remove and place on vacuum pump
- Measure dimensions of microbial mat section
- Using spatula, carefully scrape all green biomaterial onto plastic weighing boat, rinsing mesh screen with DI water as necessary
- Rinse contents of weighing boat onto filtered vacuum pump using DI water until all biomass has collected on the glass filter and no liquid remains
- Remove filter and place on glass drying weight, put in microwave for 1-2 minutes, remove filter and weigh it
- Record weight and repeat until reduction in weight <5 mg
- Subtract filter weight to calculate DW of biomass, divide by area of mat to calculate density

For 4 sections of mat dried and weighed, an average biomass density of 5.4 mg/cm^2 existed on the t0 mats. See Appendix A (7.1) for calculations.

This procedure was repeated at the end of the experiment to determine rate of biomass production.

3.1.3 Evaporation and Temperature Monitoring

There were several tasks to be completed periodically each week. Due to evaporation rates of 1-1.5 cm/24hr in the CO_2 fed tanks, and ~0.5-1 cm/24hr in the $NaHCO_3$ fed tanks, addition of DI water was necessary at least every other day to ensure proper functioning of the pumping and heating systems. The difference in humidity between the two conditions accounts for the disparity in evaporation rate.

The temperature of each tank was periodically checked to ensure optimum microalgae productivity.

3.1.4 pH monitoring

Using an Adwa AD1030 pH/mV meter, pH was checked throughout the experiment to ensure a range of 6-10. This not only represents ideal conditions for the microbial mat, but also ensured that the bicarbonate would not precipitate due to low pH.

3.1.5 CO_2 concentration and Flow Monitoring

The concentration of the CO_2 feed was routinely monitored via Witt Oxybaby M+ meter and a flow meter. A range of 2.0-2.3% CO_2 at a flow rate of 0.75-1 L/min was considered optimal operating conditions for the system. The CO_2 concentration and flow were recorded weekly.

3.1.6 Nitrate Monitoring

Nitrate levels were tested via 3- and 4-fold dilutions to remain within the equipment range. The testing equipment was a Merck RQflex and the reportable range of the testing strips used was 5-225 mg/L NO_3^-. See Appendix B for instrumental specs.

- Set testing code for RQflex
- Dip test strip in diluted sample for 2 seconds, while simultaneously hitting the "test" button on the instrument
- Remove strip, slough excess water off by touching side of strip to paper towel
- Let sit face up for 60 seconds
- Place test strip in analysis slot, pulling light-tight hinged door to the side
- Wait for displayed value
- Multiply to account for dilution

3.1.7 Nitrite Monitoring

Nitrite levels were tested via 3- and 4-fold dilutions to remain within the equipment range. The testing equipment was a Merck RQflex and the reportable range of the testing strips used was 0.5-25 mg/L NO_2^-. See Appendix B for instrumental specs.

- Set testing code for RQflex
- Dip test strip in diluted sample for 2 seconds, while simultaneously hitting the "test" button on the instrument
- Remove strip, slough excess water off by touching side of strip to paper towel
- Let sit face up for 15 seconds
- Place test strip in analysis slot, pulling light-tight hinged door to the side
- Wait for displayed value
- Multiply to account for dilution

3.2 Analysis and Design

The design process involved reaction rate modeling, graphical analysis of theoretical and experimental values, and subsequent experimental outline to improve accuracy of prototype.

First order reaction rates were assumed for the system, and using the equation

$$\ln\left(\frac{C}{C_0}\right) = -kt$$

The rate constant k was identified. Theoretical nitrate levels using maximum nitrate concentration, minimum concentration, and the calculated rate constant were plotted with experimental concentrations measured from t0 (the first running day of the experiment) to t35 (the last running day of the experiment).

Further experimentation was proposed to identify additional data points necessary for differential equation modeling.

4 Results

4.1 Precipitate Formation

Upon addition of solid $NaHCO_3$ a solid white precipitate was seen forming on the water pumps, heaters, aquarium glass, and surface of the water. At 35 C, $NaHCO_3$ has a solubility of >96 g/L in water, indicating the presence of additional ions in the sample. A small-scale recreation of tank conditions was prepared in a 500 ml glass beaker and analyzed.

4.1.1 Precipitate Experimentation and Lab Analysis

The formation of a solid white precipitate occurred in a bench-scale recreation of $NaHCO_3$ tank conditions. Figures _ and _ show the precipitate forming the tank and beaker, respectively. Upon testing the pH of the affected tank and beaker, it was determined that further lab analysis was required to determine the source of precipitation (section 3.1.1). Lab results revealed an existing dissolved Ca^+ concentration of 890 mg/L, 500 mg/L Mg^+, and a salinity of 10 EC. (See Appendix A). All dissolved compounds in the wastewater were determined to be within the range of maximum concentration of instrument interference when measuring nitrate/nitrite. (Appendix B).

4.2 Nitrate Removal

The average of the nitrate levels for both tank conditions were calculated to compare removal capabilities/differences between the control mats (CO_2) and experimental mats ($NaHCO_3$). As seen in Figure 11 below, the microbial mats in both tank conditions performed similarly, with <15 ppm difference between the averaged values for each testing interval. During the first testing interval, multiple electrical problems were identified and resolved. Steady-state conditions (continuous flow, volume, temperature) were not met until t7.

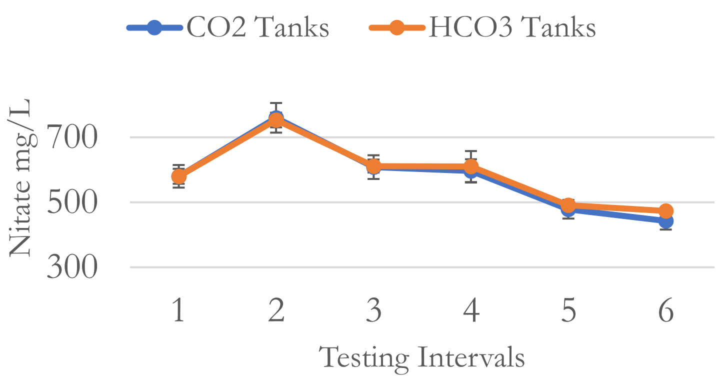
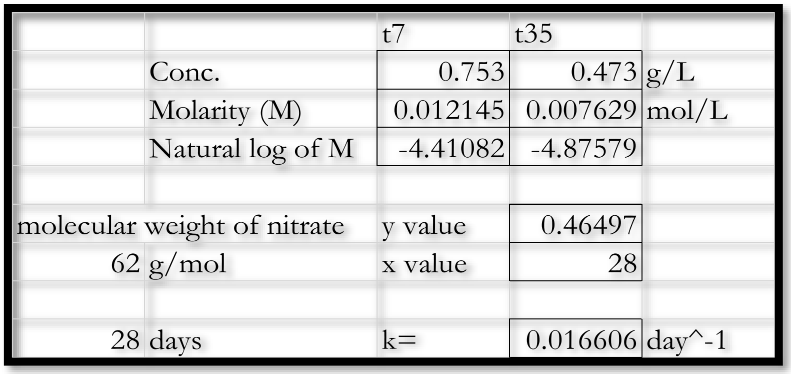


Figure 11. Average nitrate ppm for CO2 and HCO3 tank conditions (t0-t35)

The table below shows the excel calculations used to identify a rate constant, k. Using molarity and the natural log, the data showed that k=0.0166/day.



		t7	t35	
	Conc.	0.753	0.473	g/L
	Molarity (M)	0.012145	0.007629	mol/L
	Natural log of M	-4.41082	-4.87579	
molecular weight of nitrate		y value	0.46497	
62	g/mol	x value	28	
28	days	k=	0.016606	day^-1

These values were then plotted with the averaged experimental values for $NaHCO_3$ tanks to compare modeling accuracy:

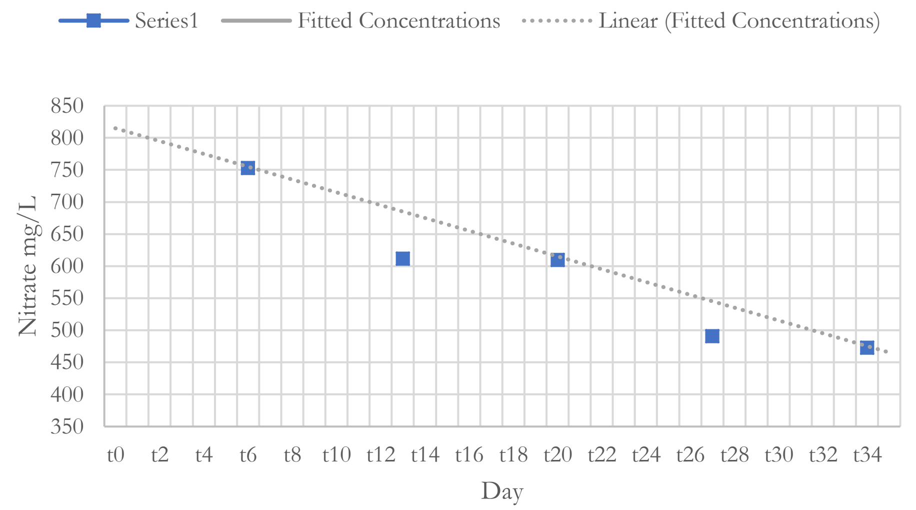


Figure 12. NaHCO3 Tanks Average Tested Nitrate ppm and Fitted Reaction-Rate Concentrations

Crossover with three experimental data points confirmed that the reaction rate modeling described the experimental system with reasonable accuracy.

4.2.1 Removal efficiency

From t0 to t35, the CO_2 tanks averaged a 23.7% removal efficiency for nitrate. The $NaHCO_3$ tanks averaged 18.4% removal efficiency. However, the spike of nitrate production accounted for a heavier nutrient load on the mats and excess nitrogen to accumulate. The removal efficiencies for the CO_2 and $NaHCO_3$ from t7 nitrate levels were 41.8% and 37.2%, respectively. Calculations for removal efficiencies can be found in Appendix C.

Average weight of nitrate assimilated per cm^2 of mat was calculated as follows:

Average mg NaHCO3 t7-t35 reduction per sq. cm of mat
280 mg/L
1120 mg/28 days
40 mg/day
0.635 mg/cm^2

Additional experimentation with increased surface area is required to accurately relate the change in surface area with the change in reaction rate, if such a relationship exists.

4.3 Nitrite Production and Removal

The process of denitrification, as detailed in section 2.5, can be identified by the timed production of nitrite in concurrence with the reduction of nitrate. Figures 13 and 14 show the production and reduction of nitrite species in both experimental and control conditions.

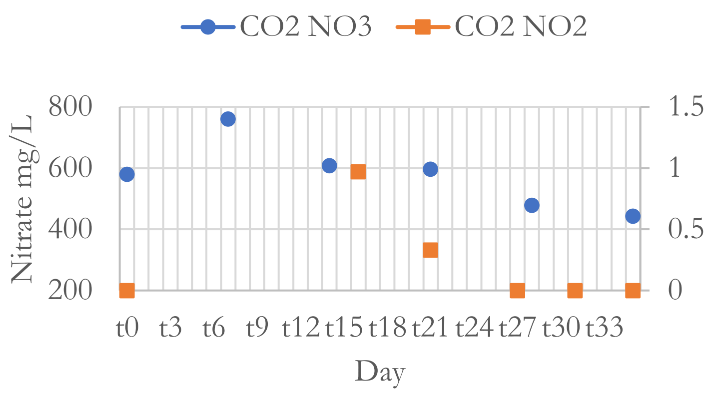


Figure 13. CO2 Tanks Average Nitrite and Nitrate ppm over time

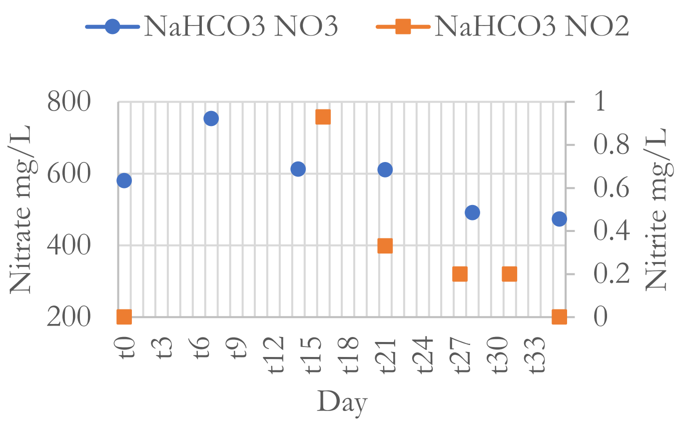


Figure 14. NaHCO3 Tanks Average Nitrite and Nitrate ppm over time

4.4 Biomass Production

T0 biomass was calculated by measuring samples of the source mat and averaging the dried weights. Average biomass was calculated to be 5.423 mg/cm^2. Due to the sublimation of precipitated $CaCO_3$ in between filamentous layers of the $NaHCO_3$ treated mats, DW was affected by the additional mass and skewed biomass growth data. Upon completion of the experiment, no definitive growth patterns were identified for the $NaHCO_3$ matts.

4.4.1 Morphological Comparisons and Growth Patterns between Experimental Groups

Throughout the experiment, numerous community shifts were observed on the $NAHCO_3$ fed mats. Figures 15 and 16 below show a morphological comparison of the CO_2 fed mats and the $NaHCO_3$ fed mats. Bleaching, browning, and detachment occurred on sections of the $NaHCO_3$ mats throughout the experiment. However, new green filamentous growth would occur after each die-off, indicating increased survivability for one or more species on the mat.[18]

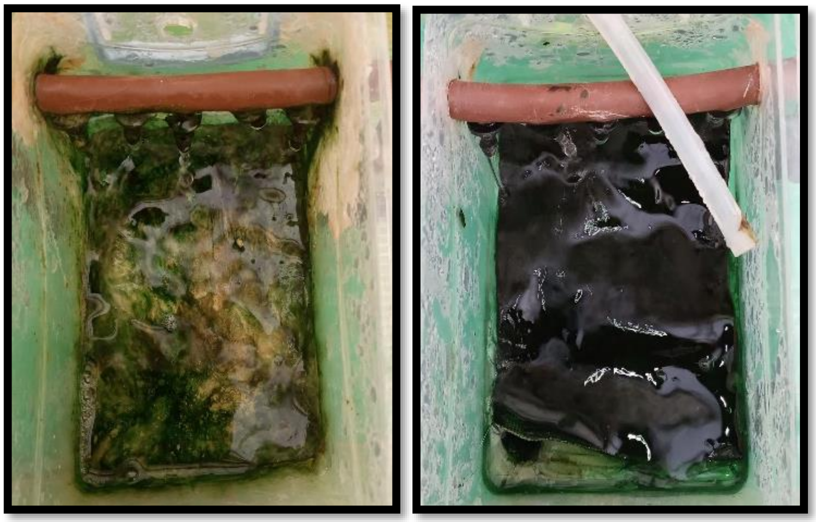


Figure 15 (left). $NaHCO_3$ fed microbial mat; Figure 16 (right). CO_2 fed microbial mat

Figure 17. The three experimental $NaHCO_3$ mats (left) and control CO_2 mats (right) on t35.

After the Ca$^+$ ions precipitated out and more HCO_3 remained in solution, green growth occurred on all three $NaHCO_3$ mats and, while lighter green in color, showed equal surface area coverage and similar thickness to the CO_2 mats. Additional genetic testing of the mats would be necessary to determine relative abundance of HCO_3 capture tolerant microorganisms.

5 Conclusions and Recommendations

5.1 Efficacy of $NaHCO_3$ as a Carbon Source

Based on the maximum removal efficiencies of the CO_2 mats (41.8%) and the $NaHCO_3$ mats (37.2%), it is evident that $HaHCO_3$ provides a viable carbon source for cyanobacterial-algal mat bioproductivity. New filamentous growth was observed throughout the experiment (t0-t35) on $NaHCO_3$ fed mats, indicating support of photosynthetic processes for >1 denitrifying species present.

5.2 Efficacy of Microbial Mats as a Wastewater Treatment Technology

The findings of this experiment support the theory that microbial mats have the potentiality to be applied as a wastewater treatment technology for water recycling and reuse purposes. Reaction modeling revealed a single rate constant (0.166/day) for the average of the $NaHCO_3$ tanks, however differential equation modeling to describe the change in rate constant as a result of nutrient loading capabilities per square centimeter of mat is impossible without additional data. The scope of potential application is currently limited by the constraints of this individual lab-bench experiment: further experimentation is required

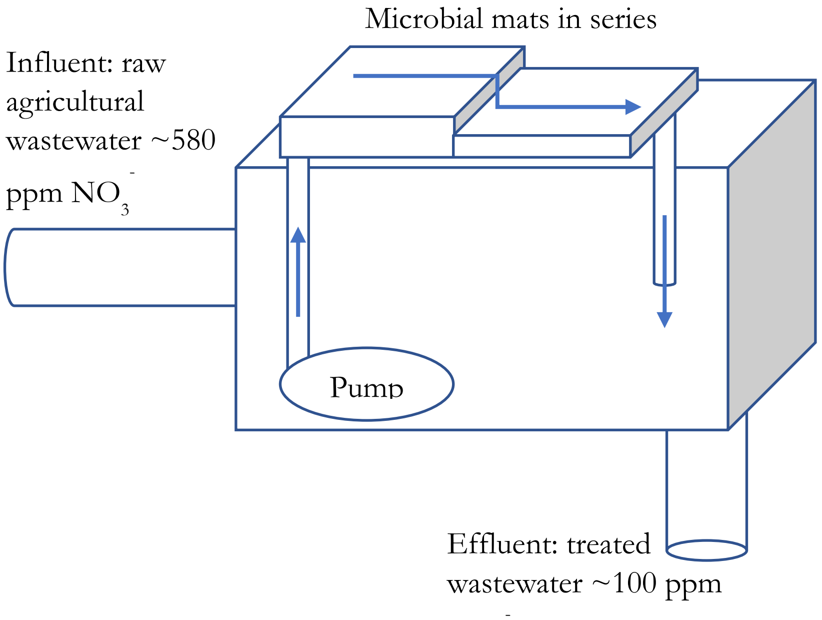
5.3 Prototype Design: Batch Bioreactor

Integrating the analyzed data to a prototype design involved modeling the system as a batch bioreactor with a hydraulic residence time (HRT) calculated by setting desired removal efficiency and using the identified rate constant. The prototype incorporates the same pumping and continuous flow over the surface of the microbial mats. Experiment 2 (section 5.4), will determine the appropriate reaction rate modeling for increased mat surface area. The system would be able to adapt for increased volume by having multiple bioreactors in series.

5.3.1 Design Specifications

The following pilot prototype was developed using reaction rate modeling and using a goal of an effluent concentration of 100 ppm nitrate. While this is still outside acceptable range for groundwater recharge, the effluent could be recycled to re-water the date tree crops, as they have higher tolerances for nutrient loading. A UV-resistant plastic tank with a 10 liter capacity would serve as the bioreactor material, with PVC pipe diameters of 5 inches to reduce turbulent flow in and out of the reactor. Plastic should be used for surfaces with the potential to be exposed to $CaCO_3$ buildup, as scouring with acetic acid may be necessary. A pump size capable of producing a maximum Q= 1000 L/hr (double the capacity of the experimental pumps describe in section 3.1). Microbial mats in series would accommodate increased batch volume.

$$C = C_0 * e^{-0.0166t}$$



Batch Bioreactor

HRT: 52 days

Surface area: 126 cm^2

C_0 = 580 ppm NO_3

C = 100 ppm NO_3

5.4 Recommendations for Further Experimentation

Due to the three-dimensional nature of microbial mat growth, further experimentation is required to accurately relate reaction rate constants with increasing surface area (it is currently unknown if the correlation is linear or exponential). The following experimental designs might serve as a means for increased accuracy of reaction rate modeling and ultimately a realized mechanistic prototype design:

Experiment 2

- Varying surface area of microbial mats being treated with both CO_2 and $NaHCO_3$
  - Eight tanks per experimental group
  - Same conditions as Experiment 1 [MQP] (4 liters wastewater, 35 C, continuous flow)
  - Average the concentrations of two identical tanks per experimental group
  - Calculate rate constants for each average, differentiate to derive empirical equation that correlates surface area to reaction rate
  - Determine HRT for 4 liter batch reactor to achieve effluent of >100 ppm

	CO_2	$NaHCO_3$
Tank 1, 2	80 cm^2	80 cm^2
Tank 3, 4	100 cm^2	100 cm^2
Tank 5, 6	120 cm^2	120 cm^2
Tank 7, 8	140 cm^2	140 cm^2

Experiment 3

- Increased volume of wastewater (increased Q)
  - Using HRT and differential equation modeling from Experiment 2, scale system for increased flow modeling
  - Constant microbial mat surface area, increasing batch reactor volume

	$NaHCO_3$
Batch reactors 1, 2, 3	8 liters
Batch reactors 4, 5, 6	12 liters
Batch reactors 7, 8, 9	18 liters
Batch reactors 10, 11, 12	27 liters

Experiment 4

- Using optimal volume batch reactor/microbial mat surface area, determine the potential role artificial UV light could play in increased bioproductivity
  - Light cycle manipulation
  - Cost benefit analyses

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7 Appendices

7.1 Appendix A

7.1.1 Biomass density calculations

Sample ID (cm x cm)	Tare (Glass Filter) mg	Dry weight (mg)	Net (mg)	Area (cm^2)
3.8 x 2.3	80.1	132.2	52.1	8.74
3.8 x 2.0	81.1	122.7	41.6	8.64
3.2 x 2.7	80.2	122.0	41.8	7.60

Divide each sample net weight by area.

$$\frac{52.1mg}{8.74cm^2} = 5.96\frac{mg}{cm^2}$$

Take the sum of the densities and divide by three:

$$\frac{5.96 + 5.50 + 4.81}{3} = 5.423\frac{mg}{cm^2}$$

Multiple by total area of mats (7cm x 9cm):

$$63\ cm^2 * 5.423\frac{mg}{cm^2} = 341.67\ mg\ average\ Biomass\ per\ mat$$

7.1.2 Laboratory analysis of wastewater:[21]

החברה הכלכלית למ.א. בני שמעון

מעבדת שרות השדה גילת

מחוז הדרום

תוצאות בדיקות מים ותמיסות עבודה - 42998

לכבוד 13487
גבי בנט
13487
יהל 88850
gabi@adssc.org
1158/0005 הערה מ.ה. -18שליח

בעבודה זו מדגם אחד בלבד
מוסר המדגמים שליח
טלפון 6355963
טל נייד 052-8835997

יג באדר א תשעט
18/02/2019
תאריך קבלה: **05/02/19**
שעת קבלה: 08:00
תאריך דיגום: **00/00/00**
תאריך מסירת תוצאות: **13/02/19**
דף: 1 0.01

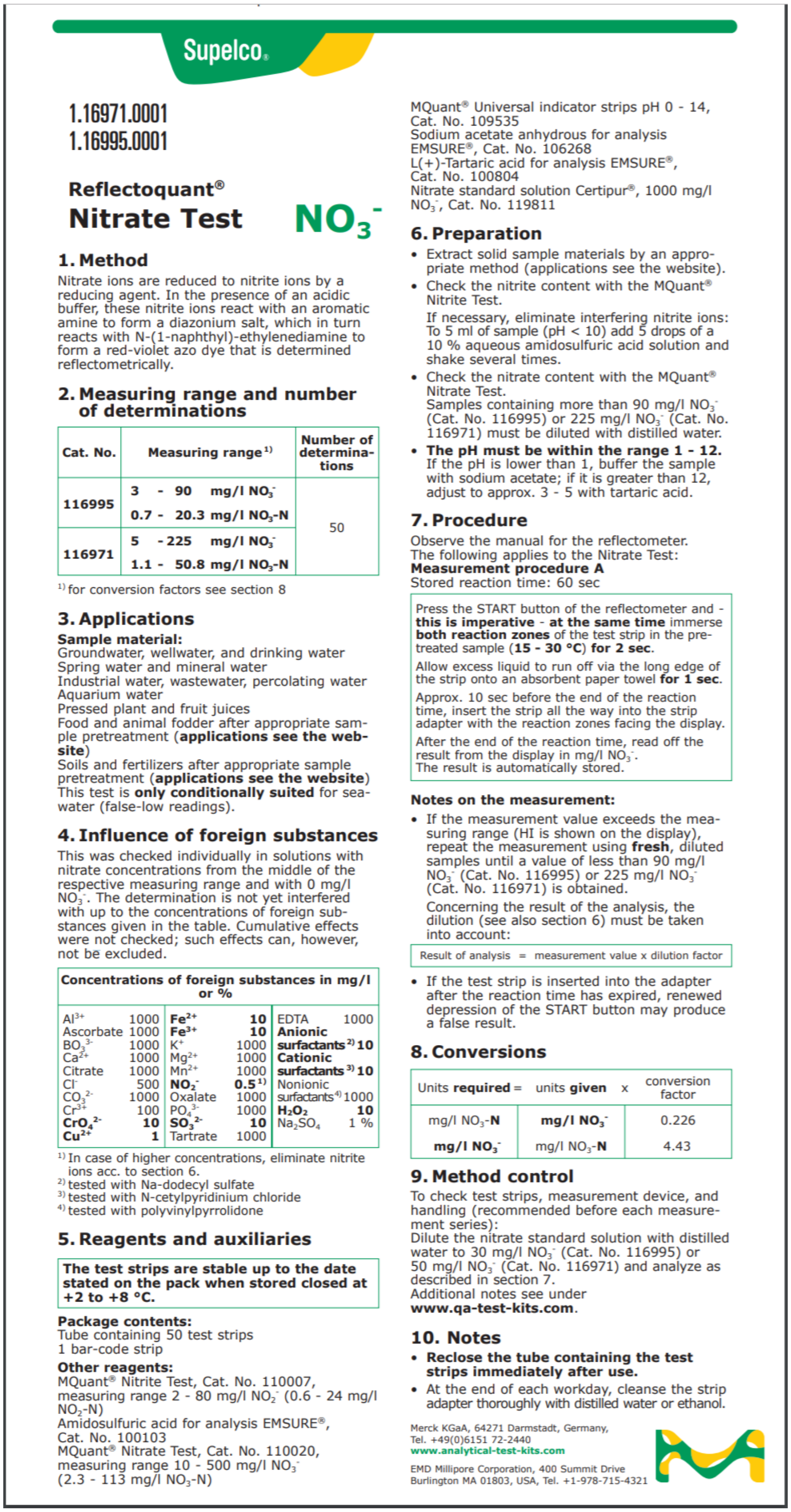
מדגם **1** חלקה **מי נקז** גידול/זן/כנה:
החומר הנבדק: ׳נ

	הבדיקה	יחידות		שיטה סטנדרטית	תוצאה	ה ע ר ו ת
Salinity (EC)	מוליכות חשמל	dS/m		SM-2510B	10.84	
Phosphorous	זרחן מסים	מ״ג/ל׳	mg/L	SM-3120B	0.8	
Potassium	אשלגן מסים	מ״ג/ל׳	mg/L	SM-3500 K-B	121.7	
Calcium	סידן	מ״ג/ל׳	mg/L	SM-3120B	896.00	
Magnesium	מגניון	מ״ג/ל׳	mg/L	SM-3120B	500.00	
Sodium	נתרן	מ״ג/ל׳	mg/L	SM-3500Na-B	1350.0	
	SAR	יחס	Ratio		9	

בדיקות זרחן,סידן,מגניון,אשלגן,נתרן,צורן,גפרית,בורון וסריקת מתכות הם ריכוזים כלליים(TOTAL)

7.2 Appendix B

7.2.1 Nitrate testing technical information:



Supelco

1.16971.0001
1.16995.0001

Reflectoquant®

Nitrate Test NO_3^-

1. Method

Nitrate ions are reduced to nitrite ions by a reducing agent. In the presence of an acidic buffer, these nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye that is determined reflectometrically.

2. Measuring range and number of determinations

Cat. No.	Measuring range [1]	Number of determinations
116995	**3 - 90 mg/l NO_3^-** **0.7 - 20.3 mg/l NO_3-N**	50
116971	**5 - 225 mg/l NO_3^-** **1.1 - 50.8 mg/l NO_3-N**	

[1] for conversion factors see section 8

3. Applications

Sample material:
Groundwater, wellwater, and drinking water
Spring water and mineral water
Industrial water, wastewater, percolating water
Aquarium water
Pressed plant and fruit juices
Food and animal fodder after appropriate sample pretreatment (**applications see the website**)
Soils and fertilizers after appropriate sample pretreatment (**applications see the website**)
This test is **only conditionally suited** for seawater (false-low readings).

4. Influence of foreign substances

This was checked individually in solutions with nitrate concentrations from the middle of the respective measuring range and with 0 mg/l NO_3^-. The determination is not yet interfered with up to the concentrations of foreign substances given in the table. Cumulative effects were not checked; such effects can, however, not be excluded.

Concentrations of foreign substances in mg/l or %					
Al^{3+}	1000	**Fe^{2+}**	**10**	EDTA	1000
Ascorbate	1000	**Fe^{3+}**	**10**	**Anionic surfactants [2]**	**10**
BO_3^{3-}	1000	K^+	1000	**Cationic surfactants [3]**	**10**
Ca^{2+}	1000	Mg^{2+}	1000	Nonionic surfactants [4]	1000
Citrate	1000	Mn^{2+}	1000	**H_2O_2**	**10**
Cl^-	500	**NO_2^-**	**0.5 [1]**	Na_2SO_4	1 %
CO_3^{2-}	1000	Oxalate	1000		
Cr^{3+}	100	PO_4^{3-}	1000		
CrO_4^{2-}	**10**	**SO_3^{2-}**	**10**		
Cu^{2+}	**1**	Tartrate	1000		

[1] In case of higher concentrations, eliminate nitrite ions acc. to section 6.
[2] tested with Na-dodecyl sulfate
[3] tested with N-cetylpyridinium chloride
[4] tested with polyvinylpyrrolidone

5. Reagents and auxiliaries

The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.

Package contents:
Tube containing 50 test strips
1 bar-code strip

Other reagents:
MQuant® Nitrite Test, Cat. No. 110007, measuring range 2 - 80 mg/l NO_2^- (0.6 - 24 mg/l NO_2-N)
Amidosulfuric acid for analysis EMSURE®, Cat. No. 100103
MQuant® Nitrate Test, Cat. No. 110020, measuring range 10 - 500 mg/l NO_3^- (2.3 - 113 mg/l NO_3-N)
MQuant® Universal indicator strips pH 0 - 14, Cat. No. 109535
Sodium acetate anhydrous for analysis EMSURE®, Cat. No. 106268
L(+)-Tartaric acid for analysis EMSURE®, Cat. No. 100804
Nitrate standard solution Certipur®, 1000 mg/l NO_3^-, Cat. No. 119811

6. Preparation

- Extract solid sample materials by an appropriate method (applications see the website).
- Check the nitrite content with the MQuant® Nitrite Test.
  If necessary, eliminate interfering nitrite ions: To 5 ml of sample (pH < 10) add 5 drops of a 10 % aqueous amidosulfuric acid solution and shake several times.
- Check the nitrate content with the MQuant® Nitrate Test.
  Samples containing more than 90 mg/l NO_3^- (Cat. No. 116995) or 225 mg/l NO_3^- (Cat. No. 116971) must be diluted with distilled water.
- **The pH must be within the range 1 - 12.**
  If the pH is lower than 1, buffer the sample with sodium acetate; if it is greater than 12, adjust to approx. 3 - 5 with tartaric acid.

7. Procedure

Observe the manual for the reflectometer.
The following applies to the Nitrate Test:
Measurement procedure A
Stored reaction time: 60 sec

Press the START button of the reflectometer and - **this is imperative** - **at the same time** immerse **both reaction zones** of the test strip in the pretreated sample (**15 - 30 °C**) **for 2 sec**.

Allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel **for 1 sec**.

Approx. 10 sec before the end of the reaction time, insert the strip all the way into the strip adapter with the reaction zones facing the display.

After the end of the reaction time, read off the result from the display in mg/l NO_3^-.
The result is automatically stored.

Notes on the measurement:

- If the measurement value exceeds the measuring range (HI is shown on the display), repeat the measurement using **fresh**, diluted samples until a value of less than 90 mg/l NO_3^- (Cat. No. 116995) or 225 mg/l NO_3^- (Cat. No. 116971) is obtained.
  Concerning the result of the analysis, the dilution (see also section 6) must be taken into account:

Result of analysis = measurement value x dilution factor

- If the test strip is inserted into the adapter after the reaction time has expired, renewed depression of the START button may produce a false result.

8. Conversions

Units **required** =	units **given** x	conversion factor
mg/l NO_3-**N**	**mg/l NO_3^-**	0.226
mg/l NO_3^-	mg/l NO_3-**N**	4.43

9. Method control

To check test strips, measurement device, and handling (recommended before each measurement series):
Dilute the nitrate standard solution with distilled water to 30 mg/l NO_3^- (Cat. No. 116995) or 50 mg/l NO_3^- (Cat. No. 116971) and analyze as described in section 7.
Additional notes see under **www.qa-test-kits.com**.

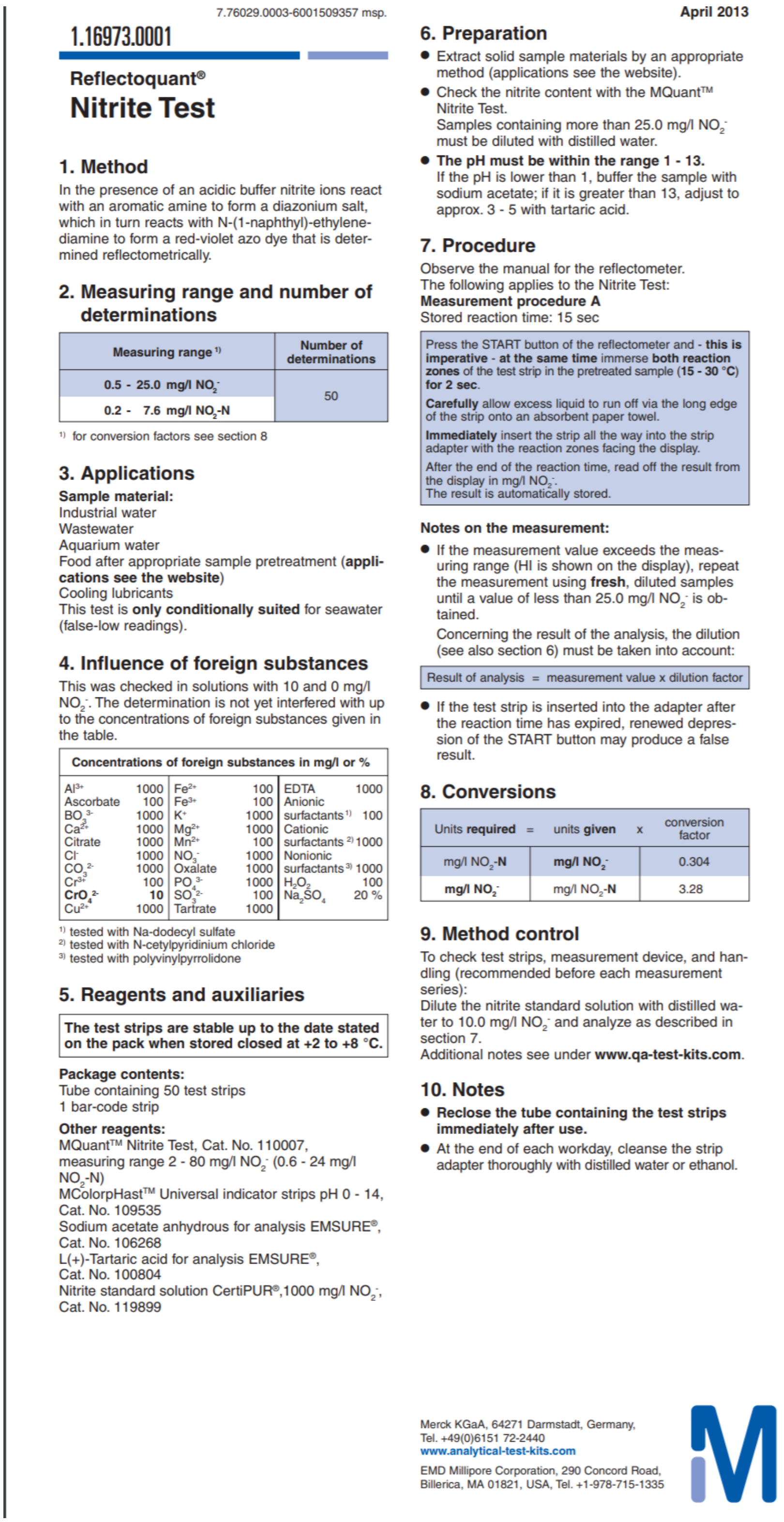
10. Notes

- **Reclose the tube containing the test strips immediately after use.**
- At the end of each workday, cleanse the strip adapter thoroughly with distilled water or ethanol.

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.analytical-test-kits.com

EMD Millipore Corporation, 400 Summit Drive
Burlington MA 01803, USA, Tel. +1-978-715-4321

7.2.2 Nitrite testing technical information:



7.76029.0003-6001509357 msp.

April 2013

1.16973.0001

Reflectoquant®

Nitrite Test

1. Method

In the presence of an acidic buffer nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye that is determined reflectometrically.

2. Measuring range and number of determinations

Measuring range [1]	Number of determinations
0.5 - 25.0 mg/l NO_2^-	50
0.2 - 7.6 mg/l NO_2-N	

[1] for conversion factors see section 8

3. Applications

Sample material:
Industrial water
Wastewater
Aquarium water
Food after appropriate sample pretreatment (**applications see the website**)
Cooling lubricants
This test is **only conditionally suited** for seawater (false-low readings).

4. Influence of foreign substances

This was checked in solutions with 10 and 0 mg/l NO_2^-. The determination is not yet interfered with up to the concentrations of foreign substances given in the table.

Concentrations of foreign substances in mg/l or %					
Al^{3+}	1000	Fe^{2+}	100	EDTA	1000
Ascorbate	100	Fe^{3+}	100	Anionic	
BO_3^{3-}	1000	K^+	1000	surfactants [1]	100
Ca^{2+}	1000	Mg^{2+}	1000	Cationic	
Citrate	1000	Mn^{2+}	100	surfactants [2]	1000
Cl^-	1000	NO_3^-	1000	Nonionic	
CO_3^{2-}	1000	Oxalate	1000	surfactants [3]	1000
Cr^{3+}	100	PO_4^{3-}	1000	H_2O_2	100
CrO_4^{2-}	**10**	SO_3^{2-}	100	Na_2SO_4	20 %
Cu^{2+}	1000	Tartrate	1000		

[1] tested with Na-dodecyl sulfate
[2] tested with N-cetylpyridinium chloride
[3] tested with polyvinylpyrrolidone

5. Reagents and auxiliaries

The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.

Package contents:
Tube containing 50 test strips
1 bar-code strip

Other reagents:
MQuant™ Nitrite Test, Cat. No. 110007, measuring range 2 - 80 mg/l NO_2^- (0.6 - 24 mg/l NO_2-N)
MColorpHast™ Universal indicator strips pH 0 - 14, Cat. No. 109535
Sodium acetate anhydrous for analysis EMSURE®, Cat. No. 106268
L(+)-Tartaric acid for analysis EMSURE®, Cat. No. 100804
Nitrite standard solution CertiPUR®,1000 mg/l NO_2^-, Cat. No. 119899

6. Preparation

- Extract solid sample materials by an appropriate method (applications see the website).
- Check the nitrite content with the MQuant™ Nitrite Test.
  Samples containing more than 25.0 mg/l NO_2^- must be diluted with distilled water.
- **The pH must be within the range 1 - 13.**
  If the pH is lower than 1, buffer the sample with sodium acetate; if it is greater than 13, adjust to approx. 3 - 5 with tartaric acid.

7. Procedure

Observe the manual for the reflectometer.
The following applies to the Nitrite Test:
Measurement procedure A
Stored reaction time: 15 sec

Press the START button of the reflectometer and - **this is imperative** - **at the same time** immerse **both reaction zones** of the test strip in the pretreated sample (**15 - 30 °C**) **for 2 sec**.

Carefully allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel.

Immediately insert the strip all the way into the strip adapter with the reaction zones facing the display.

After the end of the reaction time, read off the result from the display in mg/l NO_2^-.
The result is automatically stored.

Notes on the measurement:

- If the measurement value exceeds the measuring range (HI is shown on the display), repeat the measurement using **fresh**, diluted samples until a value of less than 25.0 mg/l NO_2^- is obtained.
  Concerning the result of the analysis, the dilution (see also section 6) must be taken into account:

Result of analysis = measurement value x dilution factor

- If the test strip is inserted into the adapter after the reaction time has expired, renewed depression of the START button may produce a false result.

8. Conversions

Units required	=	units given	x	conversion factor
mg/l NO_2-**N**		**mg/l NO_2^-**		0.304
mg/l NO_2^-		mg/l NO_2-**N**		3.28

9. Method control

To check test strips, measurement device, and handling (recommended before each measurement series):
Dilute the nitrite standard solution with distilled water to 10.0 mg/l NO_2^- and analyze as described in section 7.
Additional notes see under **www.qa-test-kits.com**.

10. Notes

- **Reclose the tube containing the test strips immediately after use.**
- At the end of each workday, cleanse the strip adapter thoroughly with distilled water or ethanol.

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.analytical-test-kits.com

EMD Millipore Corporation, 290 Concord Road,
Billerica, MA 01821, USA, Tel. +1-978-715-1335

7.3 Appendix C

7.3.1 Removal efficiency calculations

$$\% \, Removal = \frac{Initial - Final}{Initial} * 100\%$$

Calculations in Excel provided the following removal efficiencies before the spike in nitrate concentration and after.

	Removal Efficiency	
	t0 - t35	t7 - t35
CO_2	23.68%	41.75%
HCO_3	18.39%	37.17%

7.3.2 Ehud Zalim email correspondence (2/13/19 -2/19/19):

What has historic testing of the lysimeter wastewater shown in nitrate content? Salinity? Dissolved minerals (Calcium, magnesium, etc)?

1. Nitrate conc. was at the beginning around 500 ppm. Salinity was around 50 ds/m. Other dissolved minerals I have to look for it, I don't remember now.

The wastewater tested in the lab had an EC of 10, is this abnormal?

2. 10 is normal, the level of salinity is decreasing with time (began around 50)

What is the capacity of the desalination plant at the farm? How many liters can it treat per day? What is the highest salinity it can treat?

3. The desalination plant can produce 120000 liters per day of desalinated water (0.9 ds/m). I don't know what ~~is~~ the highest salinity it can treat, but around 10 [is} suppose[d] to be no problem.

What is the current disposal practice of the wastewater collected in lysimeters?

4. The leaching from the lysimeter is flowing back to ground water through a cesspit.

Is MOP currently working on any wastewater recycling? If yes, please detail.

5. We don't have [a] recycling program

Where does the farm source water from? What aquifers, what wastewater, etc? What is the percentage use of each (For example, 50% of the water is pumped from wells and 50% is wastewater)?

6. The water come[s[from local wells which [are] connected to the water system. there are [a] few aquifers, confined and unconfined.