DENITRIFICATION OF WASTEWATER USING CYANOBACTERIAL-ALGAL MATS WITH SODIUM BICARBONATE

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Elisha Musgraves

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Report Submitted to:
Professor John Bergendahl, Ph.D., P.E
Professor Isa Bar-On, PhD
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/collleagues 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

1 Introduction .................................................................................................................. 8
  1.1 The State of Israel .................................................................................................. 8
    1.1.2 The Negev Region of Israel ........................................................................... 9
    1.1.3 The City of Eilat ............................................................................................ 9
    1.1.4 Kibbutzim ...................................................................................................... 9
  1.2 Soil Composition in the Arava Valley ................................................................. 10
  1.3 Groundwater and Aquifer Recharge ................................................................. 10
  1.4 Water Usage in the Negev .................................................................................... 11
    1.4.1 Israeli Desert Agriculture ............................................................................ 11
  2 Background ............................................................................................................... 12
    2.1 Agricultural Wastewater from Southern Arava R&D ...................................... 12
    2.2 Nitrate contamination ......................................................................................... 12
    2.3 Average Hours of Sunlight per Day in Israel .................................................... 12
    2.4 Redfield Ratio .................................................................................................... 12
    2.5 Denitrification ..................................................................................................... 12
    2.6 Deionized water .................................................................................................. 13
    2.7 Carbonate Uptake .............................................................................................. 13
    2.8 Microbial Mats ................................................................................................... 13
     2.8.1 Nitrogen Uptake .......................................................................................... 13
     2.8.2 Source of Microbial Mats .......................................................................... 14
     2.8.3 Fe³⁺ to enhance mat productivity ................................................................. 14
     2.8.4 Potential Alternative Wastewater Treatment Method ................................ 14
    2.9 Project Sponsor and Goals .................................................................................. 16
     2.9.1 Hypothesis .................................................................................................. 16
  3 Methodology ............................................................................................................. 17
    3.1 Bench-scale experimentation with bicarbonate .............................................. 17
      3.1.1 Calculation of Sodium Bicarbonate Weight .............................................. 19
      3.1.2 Microbial Mat Source and Biomass Calculation ........................................ 20
      3.1.3 Evaporation and Temperature Monitoring .............................................. 20
      3.1.4 pH monitoring ........................................................................................... 20
      3.1.5 CO₂ concentration and Flow Monitoring ................................................. 21
3.1.6 Nitrate Monitoring ........................................................................................................21
3.1.7 Nitrite Monitoring ........................................................................................................21
3.2 Analysis and Design ..........................................................................................................22
4 Results ..................................................................................................................................23
4.1 Precipitate Formation .........................................................................................................23
4.1.1 Precipitate Experimentation and Lab Analysis .................................................................23
4.2 Nitrate Removal ..................................................................................................................23
4.2.1 Removal efficiency ...........................................................................................................24
4.3 Nitrite Production and Removal .........................................................................................25
4.4 Biomass Production ..........................................................................................................26
4.4.1 Morphological Comparisons and Growth Patterns between Experimental Groups ...26
5 Conclusions and Recommendations ....................................................................................27
5.1 Efficacy of NaHCO₃ as a Carbon Source ...........................................................................27
5.2 Efficacy of Microbial Mats as a Wastewater Treatment Technology ...............................27
5.3 Prototype Design: Batch Bioreactor ....................................................................................27
5.3.1 Design Specifications .......................................................................................................27
5.4 Recommendations for Further Experimentation .................................................................28
6 References ............................................................................................................................30
7 Appendices ............................................................................................................................31
7.1 Appendix A .........................................................................................................................31
7.1.1 Biomass density calculations ...........................................................................................31
7.1.2 Laboratory analysis of wastewater: ..........................................................21 ..................32
7.2 Appendix B .........................................................................................................................33
7.2.1 Nitrate testing technical information: ...........................................................................33
7.2.2 Nitrite testing technical information: ...........................................................................34
7.3 Appendix C .........................................................................................................................35
7.3.1 Removal efficiency calculations ......................................................................................35
7.3.2 Ehud Zalim email correspondence (2/13/19 -2/19/19): ..................................................35
List of Figures

1. Map of the State of Israel
2. Map of Kibbutzim in the Arava Valley
3. Denotation of Hydrological Basin Unit Boundaries
4. Basic layout of lysimeter system
5. The process of denitrification
6. Microbial mat nutrient uptake diagram
7. Cyanobacteria initial relative abundance by species
8. Algae initial relative abundance by species
9. Front view of experimental layout and mechanics
10. Plan view of individual mat and tank
11. Average Nitrate ppm for CO₂ and NaHCO₃ Tank Conditions (t₀-t₃5)
12. NaHCO₃ Tanks Average Tested Nitrate ppm and Fitted Reaction-Rate Concentrations
13. CO₂ Tanks Average Nitrate and Nitrite ppm over time
14. NaHCO₃ Tanks Average Nitrate and Nitrite ppm over time
15. NaHCO₃ fed microbial mat
16. CO₂ fed microbial mat
17. The three experimental NaHCO₃ mats (left) and control CO₂ mats (right) on t₃5.
18. Batch Bioreactor Initial Prototype Design (Unscaled)
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/km².

In October 2018, the country welcomed 486,000 tourists to the state, marking the highest rate of entry for a single month. The Center Bureau of Statistics determined that an excess of 4 million tourists visited the country in 2018.

![Map of the State of Israel](image)

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. 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, 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.

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.

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

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\(^\text{13}\).

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\(^\text{14}\).

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\(^\text{21}\).

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 Hazeava 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. 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.

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.

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 \( \rightarrow 106:16:1 \). 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.

\[
NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2
\]

![Diagram of denitrification process](image)

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. The following half-reactions detail the process of denitrification of \( NO_3^- \) via \( HCO_3^- \):

\[
2NO_3^- + 12H^+ + e^- = N_2(g) + 6H_2
\]

\[
H^+ + HCO_3^- = CO_2 + H_2O
\]

\[
2NO_3^- + 13H^+ + HCO_3^- \rightarrow N_2(g) + CO_2 + 7H_2O
\]

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. The reactions can be written as:

\[
NO_3^- + 8H^+ + 8e^- \rightarrow NH_3 + 2H_2O + OH^- 
\]
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\textsubscript{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.\textsuperscript{15}

2.8.3 Fe\textsuperscript{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\textsubscript{2} and an Fe\textsuperscript{+} solution in the water would increase mat growth and nitrogen removal capabilities.\textsuperscript{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.\textsuperscript{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\textsuperscript{16}](image)

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.  

- Guangmin et al. researched bicarbonate capture of two microalgae species: *Chlorella* and *Scenedesmus obliquus*. 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%.  

- 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.
Verhovskiy’s experiments found an 81% efficiency in Total Nitrogen and \( \text{NO}_3^- \) 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/m²d removal.\(^\text{15}\)

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.\(^\text{14}\) 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 Shcharut 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 CO₂
- Plastic tubing and irrigation nozzles
- Storage tank of CO₂, Advanced Specialty Gas Equipment distribution system
- CO₂ distribution lines for control tanks
- Solid sodium bicarbonate – % pure, from manufacturer
- Deionized Water from a Zalion Conductivity Meter
- Fe³⁺ ion solution
- Merck RQflex
- Thermometer
- Merck Nitrate test strips (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+ CO₂ 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 CO₂ 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 Fe³⁺ 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, CO₂ lines were placed into a hole cut in the Tupperware. This fed a constant stream of 2.1-2.2% CO₂ air into the microbial mat headspace.

The 3 left-most tanks were not fed CO₂ air, but were treated with sodium bicarbonate, as detailed below.
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:

\[
\left( \frac{100 \text{ mg NO}_3}{62 \text{ g NO}_3 \text{ mol}} \right) \times \left( \frac{14 \text{ g N}}{\text{ mol}} \right) = \frac{23 \text{ mg N}}{L}
\]

\[
\frac{23 \text{ mg N}}{L} \times 4L = 92 \text{ mg N}
\]

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.

\[
(92 \text{ mg N}) \times \frac{106}{16} = 298 \text{ mg} \rightarrow \frac{298 \text{ mg C}}{12 \text{ g C mol}^{-1}} \times \frac{84 \text{ g HCO}_3}{\text{ mol}} = 4.3 \text{ g NaHCO}_3
\]

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

However, upon receipt 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 NaHCO₃ a solid white precipitate was seen forming on the water pumps, heaters, aquarium glass, and surface of the water. At 35°C, NaHCO₃ 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 NaHCO₃ 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 (CO₂) and experimental mats (NaHCO₃). 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.

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.
These values were then plotted with the averaged experimental values for NaHCO₃ tanks to compare modeling accuracy:

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 CO₂ tanks averaged a 23.7% removal efficiency for nitrate. The NaHCO₃ 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 CO₂ and NaHCO₃ 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 cm² of mat was calculated as follows:
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.
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/cm². Due to the sublimation of precipitated CaCO₃ in between filamentous layers of the NaHCO₃ 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 NaHCO₃ mats.

4.4.1 Morphological Comparisons and Growth Patterns between Experimental Groups

Throughout the experiment, numerous community shifts were observed on the NAHCO₃ fed mats. Figures 15 and 16 below show a morphological comparison of the CO₂ fed mats and the NaHCO₃ fed mats. Bleaching, browning, and detachment occurred on sections of the NaHCO₃ 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

After the Ca⁺ ions precipitated out and more HCO₃ remained in solution, green growth occurred on all three NaHCO₃ mats and, while lighter green in color, showed equal surface area coverage and similar thickness to the CO₂ mats. Additional genetic testing of the mats would be necessary to determine relative abundance of HCO₃ capture tolerant microorganisms.
5 Conclusions and Recommendations

5.1 Efficacy of NaHCO₃ as a Carbon Source
Based on the maximum removal efficiencies of the CO₂ mats (41.8%) and the NaHCO₃ mats (37.2%), it is evident that NaHCO₃ provides a viable carbon source for cyanobacterial-algal mat bioproductivity. New filamentous growth was observed throughout the experiment (t₀-t₃5) on NaHCO₃ 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 NaHCO₃ 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 CaCO₃ 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.
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$_3$
  o Eight tanks per experimental group
  o Same conditions as Experiment 1 [MQP] (4 liters wastewater, 35 C, continuous flow)
  o Average the concentrations of two identical tanks per experimental group
  o Calculate rate constants for each average, differentiate to derive empirical equation that correlates surface area to reaction rate
  o Determine HRT for 4 liter batch reactor to achieve effluent of >100 ppm

\[
C = C_0 \cdot e^{-0.0166t}
\]

Influent: raw agricultural wastewater $\sim$580 ppm NO$_3^-$

Effluent: treated wastewater $\sim$100 ppm

Pump

Microbial mats in series

Batch Bioreactor

HRT: 52 days

Surface area: 126 cm$^2$

$C_0 = 580$ ppm NO$_3^-$

$C = 100$ ppm NO$_3^-$
<table>
<thead>
<tr>
<th>CO₂</th>
<th>NaHCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tank 1, 2</td>
<td>80 cm²</td>
</tr>
<tr>
<td>Tank 3, 4</td>
<td>100 cm²</td>
</tr>
<tr>
<td>Tank 5, 6</td>
<td>120 cm²</td>
</tr>
<tr>
<td>Tank 7, 8</td>
<td>140 cm²</td>
</tr>
</tbody>
</table>

Experiment 3

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

<table>
<thead>
<tr>
<th>NaHCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch reactors 1, 2, 3</td>
</tr>
<tr>
<td>Batch reactors 4, 5, 6</td>
</tr>
<tr>
<td>Batch reactors 7, 8, 9</td>
</tr>
<tr>
<td>Batch reactors 10, 11, 12</td>
</tr>
</tbody>
</table>

Experiment 4

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

3. “Map of Israel. Middle East” One World – Nations Online. [site]
15. Banet, Gabi PhD Microbiology. Advisor.
21. External water analysis conducted by Gilat Field Service Laboratory, Negev Region Israel. Feb 13, 2019. gilatlab@bns.org.il
### Appendices

#### Appendix A

#### 7.1.1 Biomass density calculations

<table>
<thead>
<tr>
<th>Sample ID (cm x cm)</th>
<th>Tare (Glass Filter) mg</th>
<th>Dry weight (mg)</th>
<th>Net (mg)</th>
<th>Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8 x 2.3</td>
<td>80.1</td>
<td>132.2</td>
<td>52.1</td>
<td>8.74</td>
</tr>
<tr>
<td>3.8 x 2.0</td>
<td>81.1</td>
<td>122.7</td>
<td>41.6</td>
<td>8.64</td>
</tr>
<tr>
<td>3.2 x 2.7</td>
<td>80.2</td>
<td>122.0</td>
<td>41.8</td>
<td>7.60</td>
</tr>
</tbody>
</table>

Divide each sample net weight by area.

\[
\frac{52.1 \text{ mg}}{8.74 \text{ cm}^2} = 5.96 \frac{\text{mg}}{\text{cm}^2}
\]

Take the sum of the densities and divide by three:

\[
\frac{5.96 + 5.50 + 4.81}{3} = 5.423 \frac{\text{mg}}{\text{cm}^2}
\]

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

\[
63 \text{ cm}^2 \times 5.423 \frac{\text{mg}}{\text{cm}^2} = 341.67 \text{ mg average Biomass per mat}
\]
## Laboratory analysis of wastewater: ²¹

<table>
<thead>
<tr>
<th>ת妊ה</th>
<th>המתח</th>
<th>ייצוב</th>
<th>השפעה</th>
<th>שרפה</th>
<th>מידה</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.84</td>
<td>SM-2510B</td>
<td>סולון (EC)</td>
<td>מים וטפר</td>
<td>ג'ל סולון</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>0.8</td>
<td>SM-3120B</td>
<td>リン (PO₄)</td>
<td>מים וטפר</td>
<td>ג'לリン</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>1217</td>
<td>SM-3500 K-B</td>
<td>ס込んだ טפר</td>
<td>מים וטפר</td>
<td>ג'ל ס込んだ טפר</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>896.00</td>
<td>SM-3120B</td>
<td>סקacin</td>
<td>מים וטפר</td>
<td>ג'ל סקacin</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>500.00</td>
<td>SM-3120B</td>
<td>מים וטפר</td>
<td>מים וטפר</td>
<td>ג'ל מים וטפר</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>1350.0</td>
<td>SM-3500Na-B</td>
<td>סגול</td>
<td>מים וטפר</td>
<td>ג'ל סגול</td>
<td>מים וטפר</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>סגול</td>
<td>מים וטפר</td>
<td>ג'ל סגול</td>
<td>מים וטפר</td>
</tr>
</tbody>
</table>

 totaled by the researcher, colleagues, and the laboratory assistant.
7.2 Appendix B

7.2.1 Nitrate testing technical information:

Reflectoquant®
Nitrates Test

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 diazotized salt, which in turn reacts with N-(1-naphthyl)-ethylendiamine to form a red-violet azo dye that is determined reflectometrically.

2. Measuring range and number of determinations

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Measuring range</th>
<th>Number of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>116959</td>
<td>3 - 90 mg/l NO$_3^-$</td>
<td>50</td>
</tr>
<tr>
<td>116971</td>
<td>5 - 225 mg/l NO$_3^-$</td>
<td>1 - 50.6 mg/l NO$_3^-$</td>
</tr>
</tbody>
</table>

1) For conversion factors see section B.

3. Applications
Sample material:
- Groundwater, wellwater, and drinking water
- Spring water and mineral water
- Industrial water, wastewater, percolating water
- Aquifer 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 sea-water (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. Maximum effects were not checked; such effects can, however, not be excluded.

Concentrations of foreign substances in mg/l or %

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$</td>
<td>1000</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>1000</td>
</tr>
<tr>
<td>BO$_3^{-}$</td>
<td>1000</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>1000</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>1000</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1000</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>1000</td>
</tr>
<tr>
<td>Cl$^{-}$</td>
<td>1000</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>1000</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>1000</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>1000</td>
</tr>
<tr>
<td>Na$_2$CO$_3$</td>
<td>1000</td>
</tr>
<tr>
<td>Na$_2$Citrate</td>
<td>1000</td>
</tr>
</tbody>
</table>

1) In case of higher concentrations, analyze nitrate ions also. 
2) tested with $\text{FeCl}_3$ solution. 
3) tested with $\text{HgCl}_2$ solution. 
4) tested with potassium permanganate.

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:
- Reflectoquant® Nitrate Test, Cat. No. 110007, measuring range 2 - 80 mg/l NO$_3^-$ (0.6 - 24 mg/l NO$_3^-$)
- Nitrate reagent for analysis ESMURE®, Cat. No. 100103
- Reflectoquant® Nitrate Test, Cat. No. 110020, measuring range 10 - 500 mg/l NO$_3^-$ (2.3 - 113 mg/l NO$_3^-$)

6. Preparation
- Extract solid sample materials by an appropriate method (Applications see the website).
- Check the nitrate content with the Reflectoquant® Nitrate 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 Reflectoquant® 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 pre-
- treated 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 3 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.

8. 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 50 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 B) must be taken into account:
  - Result of analysis = measurement value × dilution factor

9. Method control
To check test strips, measurement device, and handling (recommnended 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 are under www.pq-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.

MQuant® Universal indicator strips pH 0 - 14, Cat. No. 109535
Sodium acetate anhydrous for analysis ESMURE®, Cat. No. 106268
L(+)-Tartaric acid for analysis ESMURE®, Cat. No. 100804
Nitrate standard solution Certipur®, 1000 mg/l NO$_3^-$, Cat. No. 119811

Supelco.

DND Information Corporation, 400 Summit Drive
Burlington MA 01803, USA. Tel. +1-978-715-4321
7.2.2 Nitrite testing technical information:

**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)ethylene-diamine to form a red-violet azo dye that is determined reflectometrically.

2. Measuring range and number of determinations
   
<table>
<thead>
<tr>
<th>Measuring range</th>
<th>Number of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 25.0 mg/l NO₂⁻</td>
<td>50</td>
</tr>
<tr>
<td>0.2 - 7.5 mg/l NO₂⁻ N</td>
<td>10</td>
</tr>
</tbody>
</table>

3. Applications
   **Sample material:**
   - Industrial water
   - Waste water
   - Aquarium water
   - Food after appropriate sample pretreatment (applications see the website)

4. Influence of foreign substances
   This was checked in solutions with 10 and 0 mg/l NO₃⁻. The determination is not interfered with up to the concentrations of foreign substances given in the table.

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:**
  - MQurio™ Nitrite Test, Cat. No. 110007, measuring range 2 - 80 mg/l NO₂⁻ (0.5 - 24 mg/l NO₂⁻ N)
  - MGolorPhast™ Universal indicator strips pH 0 - 14, Cat. No. 109935
  - Sodium azide anhydrous for analysis EMSURE®, Cat. No. 106028
  - Li(+)Tartaric acid for analysis EMSURE®, Cat. No. 106084
  - Nitrite standard solution Certipur®, 1000 mg/l NO₂⁻, Cat. No. 119899

6. Preparation
   - Extract solid sample materials by an appropriate method (applications see the website).
   - Check the nitrite content with the MQurio™ Nitrite Test. Samples containing more than 25.0 mg/l NO₂⁻ 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 (16 - 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 NO₂⁻. The result is automatically stored.

8. 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 NO₂⁻ is obtained.

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 NO₂⁻ and analyze as described in section 7.
    - Additional notes see under www.qe-test-kits.com.

10. Notes
    - Redose 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.
7.3 Appendix C

7.3.1 Removal efficiency calculations

\[
\% \text{Removal} = \frac{\text{Initial} - \text{Final}}{\text{Initial}} \times 100\%
\]

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

<table>
<thead>
<tr>
<th>Removal Efficiency</th>
<th>t0 - t35</th>
<th>t7 - t35</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2</td>
<td>23.68%</td>
<td>41.75%</td>
</tr>
<tr>
<td>HCO3</td>
<td>18.39%</td>
<td>37.17%</td>
</tr>
</tbody>
</table>

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 comes from local wells which are connected to the water system. There are [a] few aquifers, confined and unconfined.