Universidad Politécnica de Cartagena

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# *Denitrification of saline agricultural effluents (brine from groundwater desalination plants and agricultural leachates) in woodchip bioreactors in the SE of Spain*

*Técnicas avanzadas en investigación y desarrollo agrario y alimentario*

*Carolina Díaz García*

*Cartagena, 2021*


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Carolina Díaz García

Directores:

Dr. Juan José Martínez Sánchez

Dr. José Álvarez Rogel



Cartagena, 2021

DT-16

**CONFORMIDAD DE SOLICITUD DEAUTORIZACIÓN DE DEPÓSITO DE TESIS DOCTORAL POR EL/LA DIRECTOR/A DE LA TESIS**

D/Dª. Juan José Martínez Sánchez
Director de la Tesis doctoral
Desnitrificación de efluentes agrícolas salinos (salmueras, procedentes de desalobradoras de agua de pozo, y aguas de drenaje agrícola) mediante biorreactores de madera en el SE de España.

**INFORMA:**

Que la referida Tesis Doctoral, ha sido realizada por D/Dª. Carolina Díaz García
, dentro del Programa de Doctorado Técnicas Avanazadas en Investigación y Desarrollo Agrario y Alimentario
, dando mi conformidad para que sea presentada ante el Comité de Dirección de la Escuela Internacional de Doctorado para ser autorizado su depósito.

☑ Informe positivo sobre el plan de investigación y documento de actividades del doctorando/a emitido por el Director/Tutor (**RAPI**).

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

- ○ Ciencias
- ○ Ciencias Sociales y Jurídicas
- ◉ Ingeniería y Arquitectura

En Cartagena, a 14 de diciembre de 2020

EL DIRECTOR DE LA TESIS

JUAN JOSE|
MARTINEZ|
SANCHEZ

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Fdo.: Juan José Martínez Sánchez

**COMITÉ DE DIRECCIÓN ESCUELA INTERNACIONAL DE DOCTORADO**

DT-17

# CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL POR LA COMISIÓN ACADÉMICA DEL PROGRAMA

D/Dª. **Francisco Artés Hernández**, Presidente/a de la Comisión Académica del Programa **Técnicas avanzadas en investigación y desarrollo agrario y alimentario**

**INFORMA:**

Que la Tesis Doctoral titulada, "**Desnitrificación de efluentes agrícolas salinos (salmueras, procedentes de desalobradoras de agua de pozo, y aguas de drenaje agrícola) mediante biorreactores de madera en el SE de España**", ha sido realizada, dentro del mencionado Programa de Doctorado, por **Dª. Carolina Díaz García**, bajo la dirección y supervisión del **Dr. Juan José Martínez Sánchez**.

En reunión de la Comisión Académica, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización del Director/a de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por el Comité de Dirección de la Escuela Internacional de Doctorado.

☑ Evaluación positiva del plan de investigación y documento de actividades por el Presidente de la Comisión Académica del programa (**RAPI**).

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

- ○ Ciencias
- ○ Ciencias Sociales y Jurídicas
- ◉ Ingeniería y Arquitectura

En Cartagena, a 15 de diciembre de 2020

EL PRESIDENTE DE LA COMISIÓN ACADÉMICA

Fdo: _____ FRANCISCO DE ASIS|ARTES|HERNANDEZ

**COMITÉ DE DIRECCIÓN ESCUELA INTERNACIONAL DE DOCTORADO**

Los proyectos que han permitido la realización de esta Tesis Doctoral han sido financiados por la Cátedra de Agricultura Sostenible para el Campo de Cartagena, por el Grupo Operativo AGUAINNOVA y por la Entidad Regional de Saneamiento y Depuración de Aguas Residuales de la Región de Murcia (ESAMUR).



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En primer lugar, me gustaría dar las gracias a mis directores, Juanjo y Pepe por brindarme la oportunidad de realizar esta tesis, aunque fuera a regañadientes por mi parte. Ha sido un placer trabajar con vosotros y ver cómo dos personas aparentemente tan diferentes, son capaces de compenetrarse tan bien y hacer que las ideas surjan, una auténtica magia. No han escatimado en nada conmigo, siempre dispuestos a trabajar, ya sea en artículos o cavando. Siempre he presumido de tener estos jefes, así que es momento de plasmarlo por escrito, muchas gracias por todo.

Considero que debo agradecer este trabajo no solo a las personas que he conocido o que han estado durante el proceso de la tesis, si no a los que han estado desde el principio de los tiempos. Esto ha sido como una competición de larga distancia en aguas abiertas, por lo que comenzaré por el principio.

Desde el punto de salida esta mi familia, los que siempre han estado, están y estarán, pero en especial mis cuatro pilares. En la piragua, para que no me pierda en el camino, mis padres. Delante y marcando el ritmo Miriam, mi hermana mayor y, detrás empujando para que no me rinda Mario, el pequeño. Teniendo estos marcajes es difícil perderse o rendirse, con ellos es más fácil llegar a cualquier meta.

Sin embargo, he dicho que esto eran aguas abiertas así que habrá que pasar por varias boyas para realizar el circuito. En la primera boya tenemos a mi familia de la piscina, allí aprendí que las cosas se consiguen con esfuerzo y sacrificio, y que los estudios son lo primero (que suerte que esa lección ya la traía aprendida de casa). Con estas pautas empecé a coger ritmo.

En la segunda boya me encontré con mis amigos, los de siempre, los que conozco desde pequeña y no fallan, siempre me apoyan, aunque a veces me digan que no me han conocido sin estudiar. Con sus ánimos y su apoyo seguí progresando hacia la tercera boya.

Allí, descubrí a los amigos que he hecho en la universidad, mi familia Itopera, mis estrellitas y varios diamantes que tuve la suerte de encontrar. Siempre doy las gracias por haberlos encontrado en el camino, porque, aunque la distancia nos separa, es como si el tiempo no pasara. Siempre me animan y me comprenden, pero a veces eso no es suficiente. Llegados a este punto, en el que se apreciaba el cansancio de la competición, una vez más las circunstancias marcaban que había que seguir estudiando, así que me encaminé a la cuarta boya, la llamaban "tesis", tenía pinta de que iba a ser una transición dura.

De camino hacia la "tesis", me encontré con la zona de público, allí están todos los que han sufrido conmigo este doctorado, especialmente Antonio Peñalver, "porque juntos somos más fuertes" hemos pasado varios años de apoyo mutuo, dificultades, pero sobre todo risas y confidencias. Junto a él, están: Yolanda, Antonio Vallecillos, Héctor, José Antonio Franco, Carmen, Nazaret, Rosa, Merce, Javi, Ibo, Pedro, Cristian, Ana Vanessa, Ana Belén, Elba, Juandi, Raquel, Bryan, los profesores de la ETSIA, mis compañeros de la red de Cátedras, los alumnos de los TFG y muchos otros que siempre han tenido palabras de ánimo. En especial, me gustaría nombrar a los trabajadores de la Finca Tomás Ferro, los de la EDAR Los Alcázares y a la empresa INSAL, sin ellos no se hubieran solucionado los mil problemas y trabas que hemos tenido.

Llegados a este punto parece que he llegado a la famosa boya "tesis" pero, esto no ha terminado, ya que nada más doblar la boya se encuentra la zona de salida, que se ha convertido en la zona de avituallamiento, por lo que esto es solo la primera vuelta de la competición…

Muchas gracias a todos

## Resumen

El Campo de Cartagena (1316 $km^2$) se localiza en la Región de Murcia (sureste de España) y es una de las principales cuencas dedicadas a la agricultura intensiva de regadío en Europa. A pesar de ser un territorio de clima semiárido (temperatura media anual 18 ˚C; precipitación media anual ≈ 300 mm; evapotranspiración media anual 1275 mm), las áreas de regadío intensivo cubren actualmente entre el 30 y el 38 % de la cuenca (≈ 40 000 - 50 000 ha). Constituye uno de los principales proveedores de productos hortícolas y cítricos de los mercados europeos. Durante muchos años, los efluentes de las actividades agrícolas, así como los vertidos de plantas depuradoras de aguas residuales urbanas y filtraciones de redes de alcantarillado, se estuvieron vertiendo a las agua superficiales y subterráneas enriqueciéndolas en nutrientes, principalmente nitratos, pero también fósforo y materia orgánica. Una de las masas de agua más afectadas ha sido el acuífero Cuaternario, cuyas concentraciones de $NO_3^-$-N se encuentran entre 22 y 34 mg $NO_3^-$-N $L^{-1}$, llegando a 30 – 45 mg $NO_3^-$-N $L^{-1}$ en algunas zonas. Sin embargo, a pesar de tener mala calidad, el agua subterránea, que se extrae por medio de pozos ubicados en las propias fincas y explotaciones agrícolas, constituye uno de los principales suministros hídricos para el regadío. No obstante, para poder utilizarla es necesario desalobrarla. Este proceso, que se realiza habitualmente en desalobradoras instaladas en las explotaciones agrícolas, genera un residuo, la salmuera, altamente salino y con elevadas concentraciones de nitratos, que puede desencadenar procesos de eutrofización si se vierte a las agua superficiales y subsuperficiales. De hecho, grandes volúmenes de estas masas de agua contaminadas por nitrato descargan en el Mar Menor, bien por vía superficial o subsuperficial, aportando así toneladas de nutrientes que han perjudicado gravemente a esta laguna. Todo ello llevó a la Comunidad Europea a declarar la zona Mar Menor-Campo de Cartagena como Zona Vulnerable a la Contaminación por Nitratos bajo la Directiva 91/676/EEC.

El Mar Menor es la laguna hipersalina costera más grande de la cuenca mediterránea. Su singularidad y su elevado valor ambiental han sido reconocidos internacionalmente de modo que fue incluida en la Convención de Ramsar como humedal de importancia internacional, está declarada como Zona Especialmente Protegida de Importancia para el Mediterráneo (ZEPIM) y forma parte de la Red Natura

2000 como Lugar de Importancia Comunitaria (LIC) y como Zona de Especial Protección para las Aves (ZEPA). Durante los últimos 40 años, los vertidos de nutrientes superficiales y subterráneos a la laguna han sido principalmente efluentes y sedimentos procedentes de los campos agrícolas, vertidos ocasionales de plantas de aguas residuales y redes de alcantarillado, y descargas de salmuera de las plantas desalobradoras. Debido a los efluentes del regadío, las ramblas, que antiguamente solo llevaban agua en períodos de lluvias torrenciales, se convirtieron en cauces permanentes que aportan vertidos continuos al Mar Menor, con un caudal de más de 400 $m^3$ por año y concentraciones de nitratos superiores a 45 mg $NO_3^-$-N $L^{-1}$. Además, entre los años 2018 y 2019, se estima que ≈ 360 toneladas de $NO_3^-$-N llegaron a la laguna a través de las descargas submarinas directas de agua del acuífero Cuaternario.

En 2016, el Mar Menor sufrió una grave crisis eutrófica y la Comunidad Autónoma de Murcia impuso una nueva normativa de gestión en la zona. Entre las normas dictadas se incluyó la prohibición del uso de plantas desalobradoras por parte de los agricultores sin la previa implementación de un proceso de desnitrificación de la salmuera resultante del proceso de desalobración de las aguas subterráneas.

Considerando las abundantes evidencias que han demostrado la eficiencia de los biorreactores de astillas de madera para desnitrificar una gran variedad de tipos de efluentes, la hipótesis inicial fue que estos sistemas podrían ayudar a mitigar los problemas de contaminación por nitratos en el Campo de Cartagena. De acuerdo con esta hipótesis, el principal objetivo de la tesis fue evaluar si es factible el uso de biorreactores de astillas de madera para desnitrificar salmuera y otros efluentes salinos cargados de nitrato en el Campo de Cartagena, SE España. Hasta la fecha, sólo existen algunos trabajos que hayan estudiado la eficiencia de biorreactores para desnitrificar aguas salinas enriquecidas en nitrato y ninguno ha trabajado con salmuera procedente de plantas de desalobración. Por tanto, esta tesis se puede considerar un trabajo novedoso en el campo de las aplicaciones de biorreactores de astillas de madera en la desnitrificación.

Para alcanzar el objetivo general, se plantearon varios experimentos con objetivos específicos cuyos resultados se recogen en los capítulos 4 a 7 de la tesis:

Capítulo 4. Incluye los resultados de tres experimentos cortos realizados en modo de flujo discontinuo (llenado-vaciado) para seleccionar un sustrato orgánico adecuado para los biorreactores. El objetivo específico fue evaluar la viabilidad de la cáscara de almendra, el troceado de algarroba, el hueso de oliva y las astillas de madera de cítrico como sustratos para la desnitrificación de salmuera (conductividad eléctrica, CE ≈ 20 mS $cm^{-1}$) con elevada carga de nitrato ($NO_3^-$-N ≈ 65 - 80 mg $L^{-1}$). La eficiencia en la eliminación de nitratos y el ratio eficiencia:coste fueron analizados. Los resultados mostraron que la mejor eficiencia en la eliminación de nitratos al menor precio fue la de las astillas de madera de cítrico (3,02 ± 0,15 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ con un coste de ≈ 6 € $m^{-3}$), seguido de la cáscara de almendra (1,54 ± 0,20 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ con un coste de ≈19 € $m^{-3}$). El troceado de algarroba y el hueso de oliva no mostraron eliminación de nitratos. El troceado de algarroba generó un lixiviado ácido con una concentración de carbono orgánico soluble extremadamente alta y el hueso de oliva produjo un lixiviado muy salino. Por lo tanto, las astillas de madera de cítrico fueron el sustrato más adecuado para la desnitrificación de la salmuera. Los resultados de estos experimentos han sido publicados en el artículo científico: Díaz-García, C., Martínez-Sánchez, J.J. and Álvarez-Rogel, J. 2020. Bioreactors for brine denitrification produced during polluted groundwater desalination in fertigation areas of SE Spain: batch assays for substrate selection. Environmental Science and Pollution Research (2): 1–10. doi: https://doi.org/10.1007/s11356-020-09567-6.

Capítulo 5. Una vez se hubo seleccionado el sustrato más adecuado, se llevó a cabo un nuevo experimento, cuyos resultados se muestran en el capítulo 5. El objetivo específico fue obtener una valoración integral (incluyendo efectos de los cambios estacionales de temperatura, variaciones en la cantidad de carbono orgánico disuelto suministrado por la madera, el tiempo de retención hidráulica -TRH-, y la edad de la madera) del comportamiento y eficiencia de los biorreactores de astillas de cítrico para desnitrificar salmuera producida en una planta de desalobración. La salmuera tenía una CE ≈ 17 mS $cm^{-1}$ y una concentración de $NO_3^-$-N ≈ 48,5 mg $L^{-1}$. Se rellenaron tres biorreactores (1 $m^3$ de capacidad cada uno) con astillas de madera de cítrico que

funcionaron durante 2,5 años (121 semanas) en modo discontinuo con tres ciclos consecutivos de llenado-vaciado a la semana. En cada ciclo los biorreactores estuvieron llenos 24h. Tras el tercer ciclo, se dejaron vacíos durante 96 horas, hasta la semana siguiente que los tres ciclos de llenado-vaciado se repitieron de nuevo. La madera de cítrico aún tenía capacidad para proporcionar suficiente carbono orgánico disuelto (COD) para la desnitrificación después de las 121 semanas. La eficiencia en la desnitrificación estuvo modulada por la concentración de COD, la temperatura, el TRH y el tiempo que estuvieron los biorreactores vacíos entre ciclos de llenado-vaciado. Durante las primeras semanas, los efluentes contenían concentraciones muy altas de COD, pero posteriormente dichas concentraciones se estabilizaron. A pesar de la elevada salinidad de la salmuera, las tasas de desnitrificación fuero altas, alcanzando valores superiores al 80 % en 24 horas de TRH con temperaturas >24 °C. Los resultados de este experimento están en revisión en la revista Journal of Environmental Management. Díaz-García, C., Martínez-Sánchez, J.J., Maxwell, B.M., Franco, J.A., Álvarez-Rogel, J. Woodchip bioreactors provide sustained denitrification of brine from groundwater desalination plants. Journal of the Environmental Management. Under review.

Capítulo 6. Considerando la importancia de la temperatura en la eliminación de nitratos en los biorreactores, en el capítulo 6 se han utilizado los datos de dos trabajos anteriores (uno realizado en la Universidad Politécnica de Cartagena -UPCT, España- y otro en la Universidad de Carolina del Norte -NCSU, EEUU). El objetivo específico fue analizar en detalle el efecto de la interacción entre la calidad del carbono y la temperatura en las tasas de eliminación de nitratos en las astillas de madera. En el análisis se utilizó la edad de las astillas de madera y el tiempo desde el final de un ciclo de llenado-vaciado hasta el siguiente (o sea, el tiempo que los biorreactores están vacíos) como indicadores indirectos de la calidad y/o disponibilidad de carbono para los microorganismos. El factor que relaciona el descenso en la concentración de nitratos del agua durante la desnitrificación con el aumento de 10 °C de temperatura se denomina Q10. Este factor es, por tanto, un indicador de la sensibilidad del proceso a los cambios de temperatura. Un mayor Q10, indica que la desnitrificación responde peor a los aumentos de temperatura. Los datos mostraron que el Q10 dependió de la temperatura

y varió en función de la temperatura mínima, así como del rango total de temperatura. Los valores de Q10 en ambos experimentos variaron entre 1,8 y 3,1 y, generalmente, aumentaron al aumentar el tiempo que llevaban utilizándose las astillas de madera en los biorreactores (o sea, cuanto más tiempo lleva la madera utilizándose menos se estimula la desnitrificación al aumentar la temperatura). En los biorreactores situados en la UPCT, las tasas medias de eliminación de nitratos en el segundo año con respecto al primero disminuyeron en un 36 % en el rango de temperaturas de 10 a 15 °C y en un 7 % en el rango de 22 a 27 °C. En los biorreactores de NCSU los valores de Q10 fueron más bajos entre los días 30-287 que entre los días 480-558. Un aspecto clave fue que los valores de Q10 aumentaron al aumentar el tiempo transcurrido entre ciclos de llenado-vaciado, lo que indica que al aumentar el tiempo que los biorreactores están vacíos aumenta la eficiencia para desnitrificar en el siguiente ciclo de llenado. Todo esto sugiere que la sensibilidad del proceso de desnitrificación a la temperatura estaba relacionada con cambios a corto y largo plazo en la calidad o disponibilidad del carbono. Así, la disminución en la eficiencia de la desnitrificación a largo plazo será mayor cuando las temperaturas sean más bajas. Los resultados de este análisis han sido publicados en el artículo científico: Maxwell, B.M., C. Díaz-García, J.J. Martínez-Sánchez, F. Birgand and J. Álvarez-Rogel. 2020. Temperature sensitivity of nitrate removal in woodchip bioreactors increases with woodchip age and following drying – rewetting cycles. Environmetal Science and Water Technology (3): 3–5. doi: https://doi.org/10.1039/d0ew00507j.

Capítulo 7. En este capítulo se incluyen los resultados de un experimento a escala piloto de un año y medio operado en modo de flujo continuo. Los objetivos específicos fueron: 1) evaluar el comportamiento de los biorreactores de astillas de madera de cítrico trabajando en flujo continuo para la eliminación de nitrato de efluentes agrícolas de aguas superficiales del Campo de Cartagena; 2) obtener una primera evaluación de cómo diferentes TRH afectan a la degradación de las astillas (pérdida de peso) y de la producción de compuestos potencialmente contaminantes en los efluentes. Se usaron tres biorreactores, consistentes en tres zanjas (6 m de largo x 0,98 m de ancho x 1,2 m de profundidad) rellenas con astillas de madera de cítrico a través de las cuales se hizo pasar agua sin tratar (3 $m^3$ $d^{-1}$ en cada biorreactor) procedente de uno de los principales canales que recogen agua de drenaje agrícola y, ocasionalmente otros efluentes, del

Campo de Cartagena (canal D7). Cada biorreactor trabajó a un TRH diferente: 8, 16 y 24 h. Los TRH se fijaron variando el nivel de agua dentro de los biorreactores. Las principales características del agua a tratar fueron: pH ≈ 7,5 - 8,0, CE ≈ 5 - 8 mS $cm^{-1}$, COD ≈ 6 - 10 mg $L^{-1}$ y $NO_3^-$-N ≈ 22-45 mg $L^{-1}$. Los resultados mostraron que los biorreactores fueron altamente eficientes en la reducción de la carga de $NO_3^-$-N (TRH 8 h ≈ 56 %, TRH 16 h ≈ 75 % y TRH 24 h ≈ 88 % -valores promedio para todo el experimento-). Estos resultados fueron variando según los cambios de temperatura en las distintas estaciones del año, aumentando en los períodos más cálidos (máximo ≈ 95 - 97 % para todos los TRH) y disminuyendo en los más fríos (mínimo ≈ 12 - 41 % para todos los TRH). La pérdida máxima de peso de las astillas de madera se produjo durante los primeros seis meses en astillas situadas por encima del nivel del agua (≈ 36 %), lo que se puede atribuir a la mineralización aeróbica de compuestos orgánicos fácilmente degradables. En las astillas que se encontraron siempre bajo el agua, la pérdida de peso fue ≈12 %. Aunque, en general, las concentraciones de sulfuro, amonio y fósforo reactivo disuelto en los efluentes fueron bajas, se produjeron picos de altas concentraciones. Las emisiones de $CO_2$ tendieron a alcanzar los valores más altos y con mayor variabilidad en el biorreactor de 8 h de TRH (≈ 714 mg $CO_2$ $m^{-2}$ $h^{-1}$; máx. = 1626; min. = 190), y las más bajas en el biorreactor de 24 h de TRH (≈ 504 mg $CO_2$ $m^{-2}$ $h^{-1}$; máx. = 926; mín. = 232). Las emisiones de $N_2O$ fueron insignificantes en los biorreactores de 8 y 16 h (< 1,7 mg $N_2O$ $m^{-2}$ $h^{-1}$), pero alcanzaron valores altos y con una muy alta variabilidad en el biorreactor de 24 h (≈ 41 mg $N_2O$ $m^{-2}$ $h^{-1}$; máx. = 168; min. = 3). Las emisiones de $CH_4$ y $NH_3$ fueron insignificantes (estos dos gases solo se detectaron en dos ocasiones con concentraciones < 0,5 mg $m^{-2}$ $h^{-1}$). Aunque los biorreactores son sistemas altamente eficientes para el tratamiento de agua enriquecida con $NO_3^-$-N en régimen de flujo continuo, se debe perfeccionar su manejo para evitar impactos ambientales debido a la presencia ocasional de compuestos nocivos en los efluentes.

La conclusión general de la tesis es que los biorreactores con astillas de madera de cítrico son sistemas adecuados para desnitrificar salmueras y otros efluentes agrícolas salinos con elevadas cargas de nitrato en el Campo de Cartagena, ya sea en flujo discontinuo o en flujo continuo. Además, las temperaturas suaves de la zona de estudio permiten una mejor eficiencia de eliminación de nitratos en un tiempo de

retención hidráulica reducido (24 horas o incluso menos) que en otros lugares con clima más frío.

Esta afirmación general se apoya en las siguientes conclusiones específicas:

1. Las astillas de madera de cítrico fue el sustrato más favorable en la desnitrificación con biorreactores en comparación con la cáscara de almendra, el troceado de algarroba y el hueso de oliva, ya que mostró las mayores reducciones de nitrato, la menor lixiviación de carbono orgánico y el menor coste económico.
2. La salinidad no impidió la desnitrificación en los biorreactores de astillas de madera.
3. Una mayor temperatura y un tiempo de retención hidráulica más prolongado favorecieron la tasa de eliminación de nitratos.
4. El envejecimiento de las astillas de madera afectó negativamente a la tasa de eliminación de nitratos, en concreto cuando la temperatura descendió por debajo de ≈ 20 ˚C. Al planificar el uso de biorreactores de madera, se debe tener en cuenta que las disminuciones de la eficiencia para desnitrificar que se producen a largo plazo serán mayores a bajas temperaturas (< 20 °C).
5. Las fases de secado de los biorreactores aumentaron la capacidad de eliminación de nitratos en las subsiguientes fases de inundación. Esto condujo a que, justo después de una fase de secado, la eficiencia se viera menos afectada por la bajada de temperatura que después de un período de inundación continua. Por lo tanto, una forma de optimizar la eliminación de nitratos durante los períodos más fríos es aumentar la frecuencia de las fases de secado entre fases de inundación.
6. Si bien las fases de secado aumentaron la eficiencia de eliminación de nitratos, también aumentaron la degradación de las astillas de madera y, por lo tanto, esto puede acortar la vida útil de los biorreactores.
7. Durante las primeras ≈ 3 - 4 semanas de funcionamiento de los biorreactores, se debe tener mucha precaución ya que se producen lixiviaciones de carbono extremadamente altas. Por lo tanto, las astillas de madera deberían lavarse antes de que los biorreactores comiencen a funcionar de forma regular. En todo caso, los efluentes deben ser controlados adecuadamente para evitar impactos

ambientales. La eficiencia en la eliminación de nitratos durante el período inicial no representa el rendimiento a largo plazo de los biorreactores.

8. Una vez que termina el lavado inicial de la madera, es habitual que las concentraciones de COD se estabilicen dentro de los niveles admisibles para el medio ambiente. Sin embargo, se pueden producir picos inesperados de alta concentración durante períodos de alta temperatura o debido a inconvenientes de operación dentro de los biorreactores.
9. Ocasionalmente pueden producirse altas concentraciones de compuestos potencialmente dañinos para la biota, como el sulfuro, durante la vida útil de los biorreactores. Para tratar de evitar esto, es necesario un control continuo de las condiciones fisicoquímicas y de la calidad del agua dentro de los biorreactores y en los efluentes. Sin embargo, dado que esta gestión puede ser difícil de implementar, medidas adicionales como el encauzamiento de los efluentes del biorreactor a humedales artificiales para eliminar compuestos indeseables distintos de los nitratos pueden ser una estrategia adecuada. De ser así, en los humedales también se eliminarían los picos de carbono orgánico de los efluentes.
10. El papel de los biorreactores de astillas de madera en la eliminación de fósforo no está claro y, por lo tanto, debe ser más investigado.
11. Los biorreactores de astillas de madera fueron una fuente de $CO_2$ y $N_2O$ (gases de efecto invernadero, GEI) a la atmósfera. El $CO_2$ se emitió principalmente cuando la mayoría de las astillas de madera estaban por encima del nivel del agua y el $N_2O$ cuando la mayoría de las astillas de madera estaban bajo el agua. Aunque se pueden proporcionar algunas pautas para tratar de reducir estas emisiones (por ejemplo, optimización del tiempo de retención hidráulica), se debe asumir que son muy difíciles de controlar de manera efectiva. Por lo tanto, la implementación de medidas de compensación por la captura de GEI podría ser una opción para equilibrar los impactos negativos de las emisiones. En este sentido, los humedales artificiales, además de actuar como amortiguadores para tratar los efluentes del biorreactor, podría contribuir a la captura de $CO_2$ y al almacenamiento de carbono.

## Summary

The Campo de Cartagena watershed (1316 $km^2$), Murcia Region, located in the southeast of Spain, is one of the main agricultural fertigation areas within Europe. Despite being a dryland territory, agricultural fertigation areas currently cover about 30 – 38% of the basin (≈ 40 000 – 50 000 ha). The area is a major supplier to European markets, particularly of horticulture products and citrus. For many years, effluents from agricultural activities, as well as from municipal wastewater treatment plants and sewage networks, have led to surface and subsurface water bodies being enriched in nutrients, mainly nitrate, but also phosphorus and organic matter. Of particular concern is the high nitrate content in the Quaternary aquifer (22 to 34 mg $NO_3^-$-N $L^{-1}$, reaching 30 – 45 mg $NO_3^-$-N $L^{-1}$ in some sectors). To support the intensive fertigation agriculture, one of the main water resources consists of groundwater withdrawal and desalinization. However, this activity implies significant environmental impacts since the brine (wastewater resulting from desalination) contains a high concentration of nitrate.

Most of the nutrients present in the water bodies have reached the Mar Menor lagoon through surface and subsurface discharges. The Mar Menor is the largest coastal hypersaline lagoon in the Mediterranean basin. It is included in the Ramsar Convention of wetlands and is a Specially Protected Area of Mediterranean Importance (SPAMI), a Site of Community Importance (SCI) and a Special Protection Area (SPA). During the last 40 years, surface and subsurface nutrient discharges to the lagoon mainly include effluents and sediments from agricultural fields, occasional discharges from wastewater treatment plants and sewage networks, and brine discharges from desalination plants. In fact, the Mar Menor lagoon in conjunction with the Campo de Cartagena were declared a Vulnerable Area to Nitrate Contamination under the Directive 91/676/EEC. The surface watercourses (called ramblas), which only carried water in periods of torrential rains, became a continuous discharge to the Mar Menor, with a flow of more than 400 $m^3$ per year and nitrate concentrations exceeding 45 mg $NO_3^-$-N $L^{-1}$. Moreover, between 2018 and 2019, it is estimated that ≈ 360 tons of $NO_3^-$-N entered the lagoon through submarine groundwater discharges from the Quaternary aquifer.

In 2016, the Mar Menor lagoon suffered a strong eutrophication crisis and the regional government implemented new management regulations. These regulations include a ban on the use of local desalination plants by farmers without implementing a brine denitrification process.

Considering the numerous worldwide evidence that have proven the effectiveness of woodchip bioreactors for denitrifying a variety of nitrate enriched effluents, the initial hypothesis of this thesis was that these systems can be an effective tool to mitigate the problem of nitrate contamination described in the Campo de Cartagena. According to this hypothesis, the main objective of this thesis was to evaluate whether the use bioreactors to denitrify brine and other saline agricultural effluents with a high nitrate load is feasible in Campo de Cartagena (Southeast of Spain). Only few works have studied the efficacy of denitrifying bioreactors for the treatment of saline water highly enriches in nitrate. Particularly, there are lack of studies denitrifying brine from desalination plants in woodchip bioreactors. Hence, this thesis can be considered a novel contribution to the state of the art of denitrifying bioreactors research.

To reach the general objective, several experiments were developed with specific objectives, which results are included in the following four chapters:

Chapter 4. This chapter includes the results of three short batch experiments performed to select a suitable carbon media for denitrifying bioreactors. The objective was to assess the viability of almond shell, chopped carob, olive bone and citrus woodchips as carbon media for denitrifying brine (electrical conductivity, EC ≈ 20 mS $cm^{-1}$) with high nitrate load ($NO_3^-$-N ≈ 65 - 80 mg $L^{-1}$). Nitrate removal efficiency and efficiency:cost ratio were considered. The results indicated that the best removal efficiency at the lowest cost was provided by citrus woodchips (3.02 ± 0.15 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ at a cost of ≈ 6 € $m^{-3}$), followed by almond shell (1.54 ± 0.20 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ at a cost of ≈19 € $m^{-3}$). Chopped carob and olive bone showed negligible nitrate removal; chopped carob generated acidic leachate with extremely high dissolved organic carbon; and olive bone resulted in highly saline leachate. Hence, citrus woodchips were the most suitable media for brine denitrification. The results of these experiments were published in the scientific paper: Díaz-García, C., Martínez-Sánchez, J.J. and Álvarez-Rogel, J. 2020. Bioreactors for brine denitrification produced during polluted groundwater desalination

in fertigation areas of SE Spain: batch assays for substrate selection. Environmental Science and Pollution Research (2): 1–10. doi: https://doi.org/10.1007/s11356-020-09567-6.

Chapter 5. Once a suitable carbon media had been selected, a new experiment was carried out, which results are shown in Chapter 5. A 2.5-years pilot scale (121 weeks) experiment was conducted in batch mode (i.e., drying-rewetting cycles). The objective was to perform a comprehensive evaluation (including effects of seasonal temperature, changes in dissolved organic carbon release, hydraulic residence time, and woodchip age) of the behavior and efficiency of woodchip bioreactors to denitrify brine from a groundwater desalination plant. The brine had electrical conductivity ≈ 17 mS $cm^{-1}$ and $NO_3^-$-N ≈ 48.5 mg $L^{-1}$. Three bioreactors (capacity 1 $m^3$ each) were filled with citrus woodchips and operated during 2.5 years in batch mode with three weekly batches of 24 hours. Citrus woodchips provided enough dissolved organic carbon (DOC) for brine denitrification even after 121 weeks of operation. Denitrification efficiency was modulated by DOC concentration, temperature, hydraulic residence time (HRT) and the drying-rewetting cycles. When DOC stabilized and temperature was > 24°C, nitrate removal efficiency was always higher than 80 %. The high salinity of brine did not hinder denitrification. The results of this experiment are under review in the Journal of Environmental Management. Díaz-García, C., Martínez-Sánchez, J.J., Maxwell, B.M., Franco, J.A., Álvarez-Rogel, J. Woodchip bioreactors provide sustained denitrification of brine from groundwater desalination plants. Journal of the Environmental Management. Under review.

Chapter 6. Considering the importance of temperature for the removal of nitrates in bioreactors, this Chapter 6 data from two previously published studies (one from the Universidad Politécnica de Cartagena -UPCT, Spain- and the other from the University of North Carolina -NCSU, EEUU-) with the objective to perform a comprehensive analysis looking at the effect of the interaction between carbon quality and temperature on nitrate removal rates in woodchips. The analysis used woodchip age and time elapsed since a DRW cycle as indicators of carbon quality/availability. The factor by which nitrate removal increased given a 10 °C increase in temperature ($Q_{10}$) was used as a metric for temperature sensitivity. $Q_{10}$ values for nitrate removal in both

experiments ranged from 1.8 – 3.1 and generally increased over time as woodchips aged. In field bioreactors, mean nitrate removal rate at temperatures 10 – 15 °C and 22 – 27 °C decreased by 36 % and 7 %, respectively, from the first to second year. $Q_{10}$ values increased with amount of time since resaturation of the woodchips following a drying-rewetting cycle. Sub setting the datasets showed that $Q_{10}$ was temperature-dependent and varied according to minimum temperature value and total range in temperature. The results suggested that temperature sensitivity of nitrate removal was related to short- and long-term changes in carbon quality or availability, according to the carbon-quality-temperature hypothesis. When sizing woodchip bioreactors, water quality managers should consider that long-term declines in efficiency will be greatest at lower temperatures. The results were published in the scientific paper: Maxwell, B.M., C. Díaz-García, J.J. Martínez-Sánchez, F. Birgand, and J. Álvarez-Rogel. 2020. Temperature sensitivity of nitrate removal in woodchip bioreactors increases with woodchip age and following drying – rewetting cycles. Environmetal Science and Water Technology (3): 3–5. doi: https://doi.org/10.1039/d0ew00507j.

Chapter 7. This chapter includes the results of a 1.5-year pilot scale experiment in continuous flow mode. The specific objectives were: 1) to assess the efficiency of citrus woodchip bioreactors working under continuous flow regime for reducing the high NO3- loads from agricultural leachates flowing in surface water courses of the Campo de Cartagena; 2) to get a preliminary assessment of how different hydraulic residence times affect woodchips degradation (weight loss) and assess the existence of potentially harmful substances in the bioreactor effluents. Three bioreactors consisting of three trenches (6 m long x 0.98 m wide x 1.2 m depth) filled with citrus woodchips through which the untreated ditch water (3 $m^3$ $d^{-1}$ per bioreactor) was routed for 1.5 years to achieve denitrification at 8 h, 16 h and 24 h hydraulic residence time (HRT) in each bioreactor, respectively. The HRT were set by varying the water level inside the bioreactors. The main characteristics of the target water were: pH ≈ 7.5 – 8.0, EC ≈ 5 – 8 mS $cm^{-1}$, DOC ≈ 6 – 10 mg $L^{-1}$, and $NO_3^-$-N ≈ 22 – 45 mg $L^{-1}$. The results showed that bioreactors were highly efficient in reducing the $NO_3^-$-N load (8 h HRT ≈ 56 %, 16 h HRT ≈ 75 % and 24 h HRT ≈ 88 % average for the entire experiment). This was modulated by seasonal changes in temperature, increasing in the warmer periods (maximum ≈ 95 - 97

% for all HRT) and decreasing in the coldest (minimum ≈ 12 - 41 % for all the HRT). The maximum woodchips weight loss accounted during the first six months (≈ 36 %) in the material just above the water level, attributable to the aerobic mineralization of easily degradable organic compounds. In the material continuously underwater woodchips weight loss was ≈ 12 %.

Although, in general, sulfide, ammonium and dissolved reactive phosphorus concentrations in the effluents were low, peaks of high concentrations occurred. The $CO_2$ emissions tended to reach the highest values with more variability in Bio8h (≈714 mg $CO_2$ $m^{-2}$ $h^{-1}$; max = 1626; min = 190), and the lowest with less variability in Bio24h (≈504 mg $CO_2$ $m^{-2}$ $h^{-1}$; max = 926; min = 232). The $N_2O$ emissions were negligible in Bio8h and Bio16h (< 1.7 mg $N_2O$ $m^{-2}$ $h^{-1}$), but they reached high values with very high variability in Bio24h (≈41 mg $N_2O$ $m^{-2}$ $h^{-1}$; max = 168; min = 3). Emissions of $CH_4$ and $NH_3$ were negligible (these two gasses were only detected on two occasions with concentrations < 0.5 mg $m^{-2}$ $h^{-1}$). Although bioreactors were highly efficient systems for treating $NO_3^-$-N enriched water under continuous flow regime, caution must be taken to avoid environmental impacts due to the occasional presence of harmful compounds in the effluents.

The general conclusion of the thesis is that citrus woodchip bioreactors are suitable systems to denitrify brine and other saline agricultural effluents with high nitrate load in the Campo de Cartagena, either under batch or continuous flow mode. Moreover, the mild temperatures in the study area allow better nitrate removal efficiency in reduced hydraulic residence time (24 hours or even less) than other places with colder climate.

This general statement is supported by the following specific findings:

1. Citrus woodchips were more favorable carbon media for denitrifying bioreactors than almond shell, chopped carob and olive bone, since they showed the highest nitrate reductions, the lowest organic carbon leaching, and had the lowest economic cost.
2. Salinity did not hinder denitrification in woodchip bioreactors.

3. Higher temperature and longer hydraulic residence time favored nitrate removal rate.
4. Woodchips aging negatively affected nitrate removal rate, particularly when temperature decreased below ≈ 20 ˚C. When planning the installation of woodchip bioreactors, it must be considered that long-term declines in denitrification efficiency will be greatest at lower temperatures (< 20 °C).
5. Bioreactors drying phases increased nitrate removal in the subsequent flooding phases. This led to just after a drying phase the efficiency was less impaired by low temperature than after a period of continuous flooding. Hence, a way to optimize nitrate removal during colder periods is to increase the frequency of alternating drying-rewetting cycles.
6. While drying phases increased nitrate removal efficiency, they also increased woodchips degradation and, therefore, may shorten bioreactors life span.
7. During the first ≈ 3 - 4 weeks of bioreactors operation much caution must be put as extremely high carbon flushes occur. Hence, woodchips must be washed before bioreactors start operating and effluents must be properly managed to avoid environmental drawbacks. Furthermore, the nitrate removal efficiency during this initial period does not represent the long-term performance of bioreactors.
8. Once finished the initial high organic carbon flush, concentrations within admissible levels for the environment are usual. However, unexpected high concentration peaks may occur during periods of high temperature or due to operation drawbacks inside bioreactors.
9. High concentrations of potentially harmful compounds for biota, such as sulfide, may occasionally occur during bioreactors life span. To try to avoid this, a continuous monitoring of the physicochemical conditions and water quality inside the bioreactors and in the effluents are necessary. However, since this management can be difficult to implement, additional measures such as routing bioreactor effluents to constructed wetlands to remove undesirable compounds

other than nitrates may be a suitable strategy. If so, organic carbon peaks in the effluents would be also removed in the wetlands.

10. The role of woodchip bioreactors in phosphorus removal is not clear and therefore deserve further research.

11. Woodchip bioreactors were a source of $CO_2$ and $N_2O$ (greenhouse gasses, GHGs) to the atmosphere. $CO_2$ was mainly emitted when most of the woodchips were above the water level and $N_2O$ when most of the woodchips were underwater. Although some guidelines can be provided to try to reduce these emissions (e.g., optimization of hydraulic residence time), it must be assumed that they are much difficult to control in an effective way. Hence, the implementation of compensation measures for capturing GHG could be an option to balance the negative impacts of the emissions. In this sense, constructed wetlands, in addition to act as a buffer to treat bioreactor effluents, could contribute to capture $CO_2$ and to carbon storage.

# Index

# Chapter 1

## Introduction and state of the art

## 1.1 Environmental issues in watersheds with intensive agricultural use

In recent years, due to increase in the world population, there has been a change in agriculture around the world to be able to supply food to that growing population. On account of the high food demand in the world, traditional agriculture has been modified towards intensive agriculture where in a smaller crop area the same amount of food can be obtained than in a large area by traditional agriculture. However, the intensity of the land use is reaching a critical level (Hefting et al., 2013). For example, to maximize the crop area farmers modify the streams by removing meanders, hardening banks and allowing agriculture to maximally use the land all the way to the stream bank (Hefting et al., 2013). In addition, intensive agriculture destroys ecosystems, alters soil structure producing soil degradation, erosion, acidification, compaction, contamination by heavy metals, and impoverishes the vegetation layer. There are also other major threats such as salinization or organic pollutants (Project Life Sinergia, 2006).

As a consequence of the environmental impacts caused by intensive agriculture, it is necessary to search for methods to alleviate these effects. These measurements should include implementation of best management practices such as crops rotation, precision agriculture, fertilizers optimization, together with environmental measures in line with nature-based solutions (NBS) such as implantation of hedges, wetlands or bioreactors for water depuration and others.

Also, intensive agriculture usually needs to apply several chemicals to maintain that high production, such as pesticides or fertilizers containing nitrate ($NO_3^-$). When leachates containing high concentrations of chemicals are washed away or infiltrate in the soil profile can pollute groundwaters or surface water bodies.

This problem about agricultural leachate pollution through the watersheds is of worldwide concern. For instance, areas affected by this threat include the Baltic Sea around Sweden, the Rhine estuary (Hefting et al., 2013) or the Gulf of Mexico (Moorman et al., 2015). A particularly of concern case occurs in arid and semi-arid zones, where there is a lack of water resources, mainly due to low rainfall. In these areas, many times is necessary withdrawal groundwater to cover the needs of the population.

This groundwater, in some cases, are polluted due to its high content in $NO_3^-$, phosphorus and other salts, organic matter, toxic compounds, heavy metals, pathogenic microorganisms, etc.

Leachates enriched in nutrients such as $NO_3^-$ and phosphorus can lead to the eutrophication of waterbodies (Blowes et al., 1994). This degrades biological, ecological, social, and economic value of the environment (Pluer et al., 2016). For this reason managing the nitrogen cycle has been identified as a Grand Challenge by the U.S. National Academy of Engineering (NAE) (Lopez-Ponnada et al., 2017), also European Union can be implemented several actions at different scales called European Water Framework Directive (2000/60/CE) in terms of water pollution by agricultural non-point source pollution (Tournebize et al., 2016).

To reduce non-point source pollution, the first essential stage is limiting the quantities of pesticides, $NO_3^-$ and other potentially harmful elements reaching aquatic environments (Tournebize et al., 2016). A second step would be to try to treat leachates, before they are discharged or once they have been discharged, but before they reach water bodies such as seas, lakes, etc.

Specifically, for $NO_3^-$ removal from effluents, different technologies have been proposed, including sequencing batch reactors with methanol as carbon substrate for denitrifier microorganisms (Clifford and Liu, 1993), upflow sludge blanket reactors (Beliavski et al., 2010), membrane bioreactor (Wisniewski et al., 2002), fluidized bed absorber reactors (Ersever et al., 2007), electrodialysis (Bosko et al., 2014), and others. Although these technologies are capable of high $NO_3^-$ reduction rates, they are not well-suited for the agricultural application in local farms because they can be expensive and technically complex to manage. Furthermore, in recent years is more relevant the fact of combined ecology systems with engineering design, which is called ecological engineering.

## 1.2 Ecological engineering for mitigating the effects of nutrient-enriched leachates

The term "ecological engineering" is defined, according to the Center for Wetland (University of Florida), as "the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both. It involves the design, construction and management of ecosystems that have value to both humans and the environment. Ecological engineering combines several basic disciplines and applied science from engineering, ecology, economics, and natural sciences for the restoration and construction of aquatic and terrestrial ecosystems." (Mitsch, 2012; International Ecological Engineering Society, 2017).

Ecological engineering solutions address an increasing demand imposed on the land surface, such as providing for water and sanitation, energy, education, nutrients, etc. but incorporating the ecological knowledge so the result is a balance between nature and development (International Ecological Engineering Society, 2017).

Many are the benefits of the ecological engineering, such as: an increase and protection of the biodiversity, producing water retention and flood protection, nutrient providing and erosion control, reduction of investment and maintenance cost, energy and resource savings, providing recreational opportunities, improving habitat for wildlife and endangering species, and providing rural prosperity and poverty reduction (International Ecological Engineering Society, 2017)(Figure 1.1).

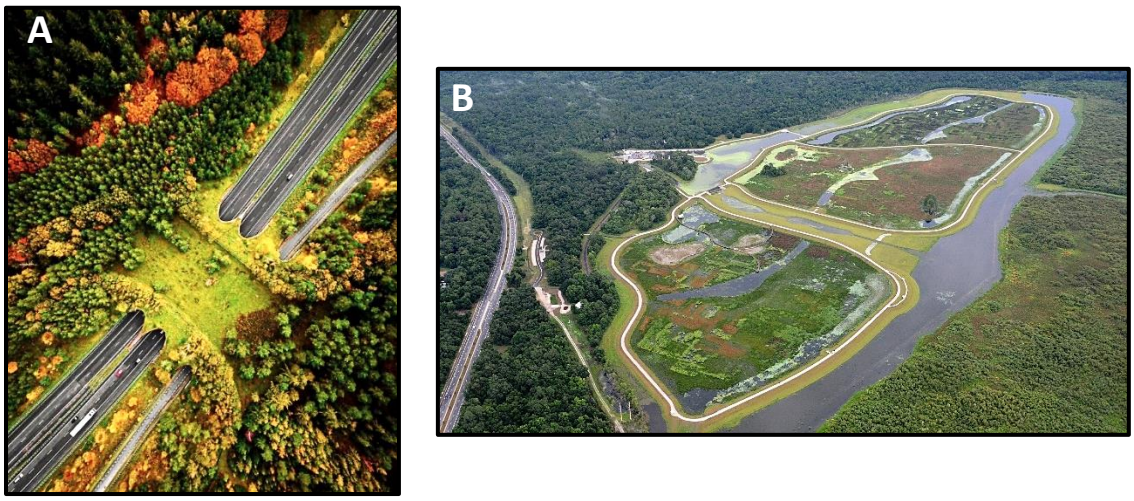


Figure 1.1. Two examples of ecological engineering. A: A crossing funnels the local wildlife safely across the highway in the Netherlands (den Hertog, 2020). B: A constructed wetland which is one of the most productive birding hotspots in Florida (Segal and Knight, 2016).

Mitsch (1998) presented six recommendations about how engineers and ecologists should work to achieve ecological engineering. In 2012, the same author revised his recommendations and concluded that there has been remarkable progress in the development of ecological engineering principles and practices but, engineers need to have a non-linear thinking and ecologists must become more active in this discipline to achieve a sustainable ecosystems that integrate human society.

In relations with ecological engineering are the implementation of NBS. The European Commission (2018) defines NBS as: "Solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience. Such solutions bring more, and more diverse, nature and natural features and processes into cities, landscapes and seascapes, through locally adapted, resource-efficient and systemic interventions". Also, NBS are determined by natural ecosystem functions involving microbial removal of pollutants from aquatic systems (Cohen-Shacham et al., 2016). These NBS are considered alternatives to man-made infrastructures that require energy and large investment in materials (Nesshöver et al., 2017).

Inside these nature-based solutions we can find some water treatment to reduce that pollution such as conservation practices for reducing $NO_3^-$ losses as wetlands (Álvarez-Rogel et al., 2020), saturated buffers (McEachran et al., 2020) and denitrification bioreactors (Moorman et al., 2015), all of them considered edge-of-field practices.

### 1.2.1 Constructed wetlands, saturated buffers and denitrifying bioreactors

Constructed wetlands are man-made areas in which the physical, chemical and biological processes for removing pollutants that normally occur in natural wetlands are reproduced in a controlled manner (Figure 1.2). During last century have increased their popularity in their application to improve water quality in different scenarios as habitat restoration, storm water runoff, sewage treatment, etc. (Zhi and Ji, 2012).

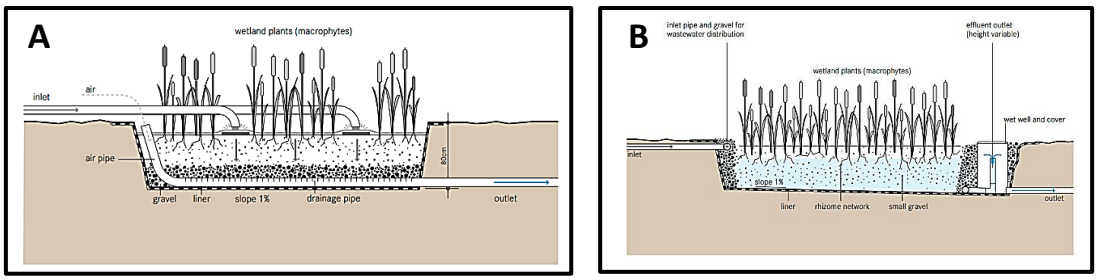


Figure 1.2. Wetlands of vertical flow (A) and wetlands of subsurface flow (B) (Tilley et al., 2014).

Saturated buffers, which intercepts tile drainage water before its outlets into a stream. The water is diverted by a water-control structure through a perforated drainage pipe and then pushed through an existing filter strip. As the water drains through the soil profile, perennial plants take up the excess nutrients (Schilling, 2019) (Figure 1.3). This technology is more used every year, for example, in the United States in January 2020, the country presented over 303 000 ha of riparian buffers enrolled in the continuous signup of the United States Department of Agriculture's Conservation Reserve Program in the states that make up the Cornbelt (USDA, 2019; Groh et al., 2020).

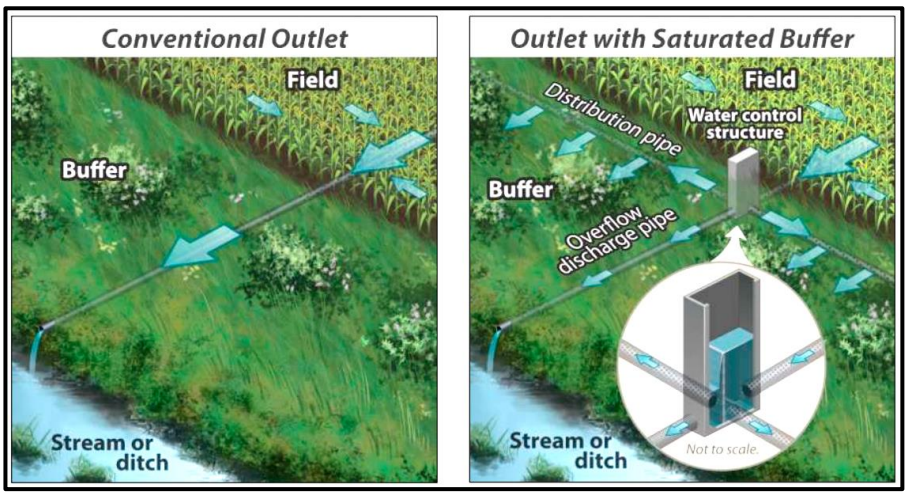


Figure 1.3. Saturated buffer scheme (Helmers and Isenhart, 2012).

Denitrifying bioreactors, which consist of trenches or containers filled with a solid carbon substrate through which $NO_3^-$-rich water is routed to achieve denitrification (Christianson et al., 2010) (Figure 1.4). In recent years have increased their application to remove $NO_3^-$ in several places around the world (Christianson and Schipper, 2016).

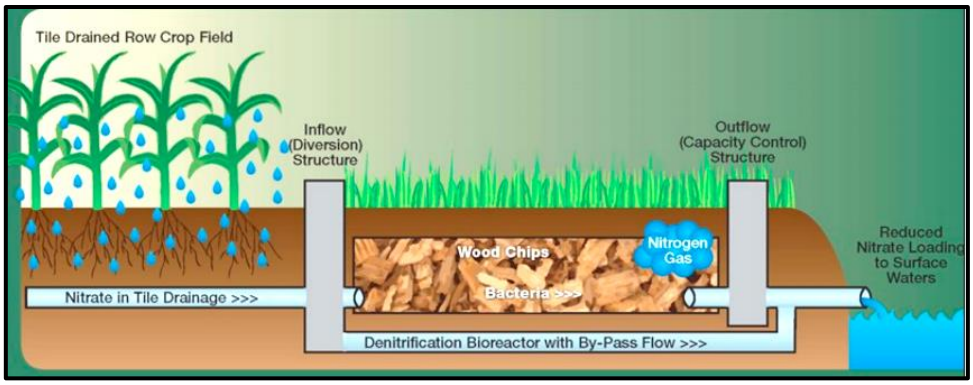


Figure 1.4. Scheme of a woodchips bioreactor (Tyndall and Bowman, 2016).

These three practices have several things in common. Have low operating costs, relatively simple to install with a reasonable cost, when all of them are constructed are relatively self-sustaining, and are highly efficient in removing $NO_3^-$ (Helmers and Isenhart, 2012). But, also have several drawbacks in common such as: require expert design and construction, a controlled drainage structure is required to divert subsurface flow to the installation (IAWA, 2015), their $NO_3^-$ removal rates dependent upon the hydraulic residence time in systems (Moorman et al., 2015), and, also the three depends on seasonality, soil/substrate type, plant species and water chemistry (Moreno et al., 2007).

The three systems have some differences among them:

- Wetlands require more space for building than denitrifying bioreactors, but also can treat water from a larger area. For instance, while bioreactors treat drainage from a field-sized area wetland receive drainage from several thousand hectares.
- Wetlands and saturated buffers can be effective for other water pollutants or sediment, while denitrifying bioreactors specifically designed for reducing water $NO_3^-$ (Tilley et al., 2014; AgBMPs, 2018).
- Wetlands and saturated buffers need to prune plants eventually, while bioreactors do not because they are unplanted.
- Bioreactors have an immediately start-up while wetlands and saturated buffers need longer time to work a full capacity (Tilley et al., 2014).

- Denitrifying bioreactors can use different types of substrates as a carbon media while wetlands and saturated buffers need perennial vegetation, that can withstand the flood, also, generated benefits for wildlife habitat and flood regulation.

## 1.3 Denitrifying bioreactors: emerging technology for $NO_3^-$ reduction in agricultural waters

Denitrifying bioreactors are passive treatment systems consist of trenches or containers filled with a carbonaceous material (typically woodchips or plant residue) through which $NO_3^-$-rich water is routed to enhance the natural process of denitrification. The carbonaceous material provides organic carbon as an electron donor for anaerobic microorganisms to complete denitrification under suboxic/anoxic conditions, where $NO_3^-$ is transformed into gaseous forms of nitrogen such as $N_2O$ or $N_2$ (Schipper et al., 2010) (Figure 1.5).

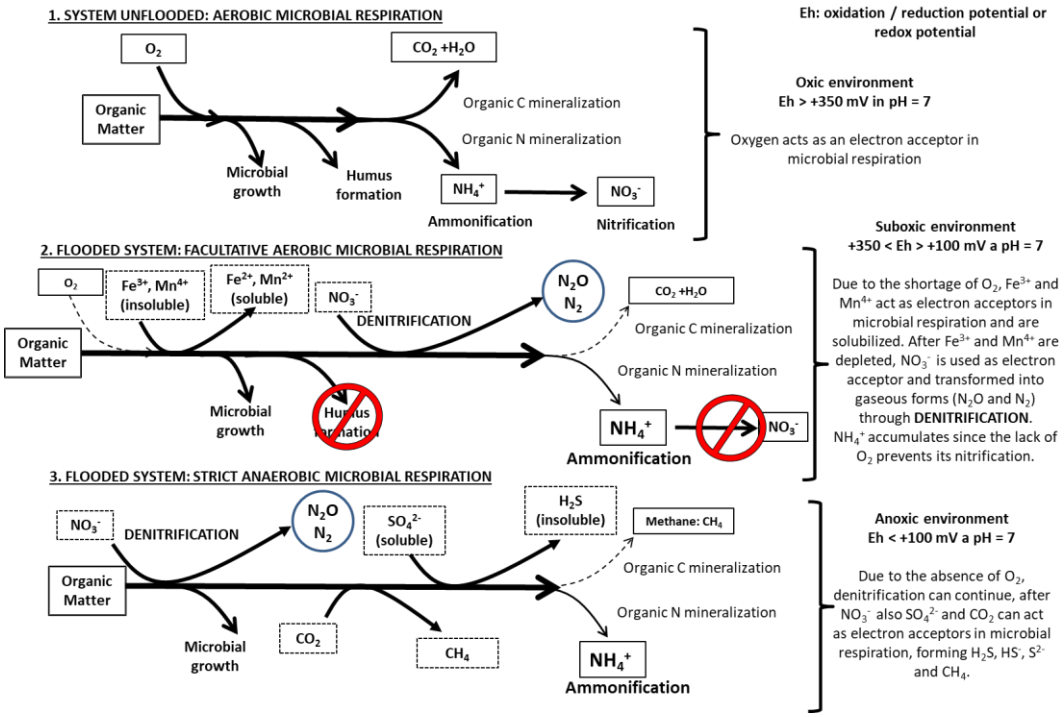


Figure 1.5. Simplified diagram of the mineralization of organic matter in systems with different flooding degrees, including N transformations and other elements and their relationship with redox potential (ORP).

Denitrification has been studied since the middle of the 20$^{th}$ century as a biological way to reduce nitrates. In 1980s some studies used biological denitrification

to reduce nitrates in groundwater using methanol or straw with algae as carbon source for microorganisms (Andreoli et al., 1980; Boussaid et al., 1988). But it was not until 1994 that denitrification bioreactors with woodchips started to use in Canada (Blowes et al., 1994).

Nowadays due to increase of $NO_3^-$ in water bodies and therefore problems such as eutrophication, denitrifying bioreactors are starting to apply all around the world, having a special involvement in Canada, New Zealand and the United States (Christianson and Schipper, 2016). Every year bioreactors are increased their value due to the use of local organic waste products, due to their simplicity because it is a passive treatment to reduce $NO_3^-$, easy to install, require low maintenance (Schipper et al., 2010; Christianson and Helmers, 2011; von Ahnen et al., 2016) and provide low-cost $NO_3^-$ removal (Christianson et al., 2009).

Bioreactors have been included in the official nutrient reduction strategies in several states in the Midwestern United States (IDALS, 2014) and in the National Service of Natural Resources of the Department of Agriculture of the United States (USDA, 2015).

### 1.3.1 Types of denitrifying bioreactors

Different types of bioreactors are used depending how the water is collected and its hydraulic connection with the bioreactor. There are three main types (Figure 1.6):

- Denitrification walls, are barrier designed to sustain elevated hydraulic conductivities which intercepting shallow groundwater where the influent is piped in or streambed bioreactors (Schipper et al., 2010; Lassiter and Easton, 2013).

- Denitrifying beds, are containers (sometimes lined) that are filled with carbon media which intercepting concentrated discharges (Schipper et al., 2010).

- Denitrifying layers, are horizontal layers of carbon media which intercepting soil leachate, in the form of a permeable reactive barrier (Robertson and Cherry, 1995; Schipper et al., 2010; Lassiter and Easton, 2013; Rivas et al., 2020).

Walls and beds denitrification are used generally for different $NO_3^-$ removal rates, beds for 2–22 g N $m^{-3}$ $day^{-1}$ and walls for 0.01 to 3.6 g N $m^{-3}$ $day^{-1}$.

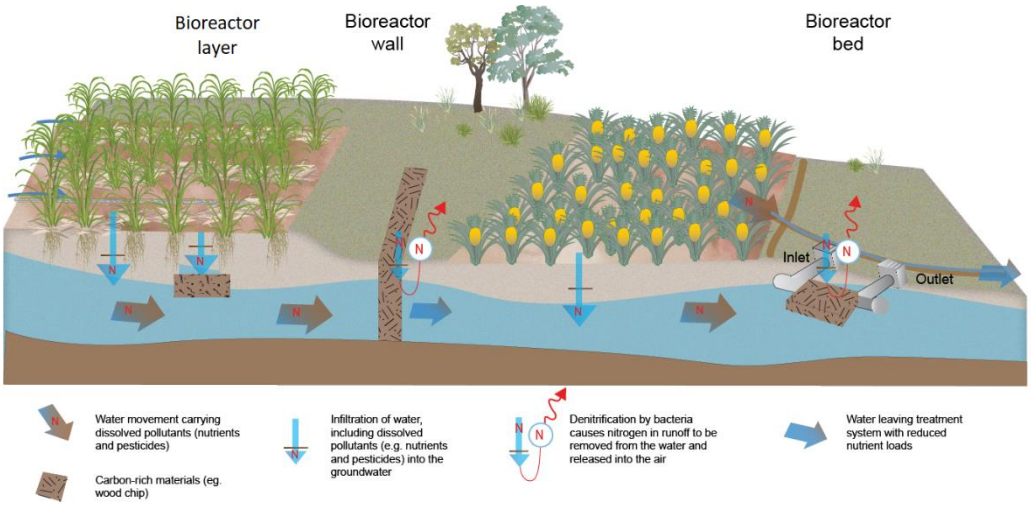


Figure 1.6. Illustration of layer, wall and bed bioreactors (Modified from Department of Environment and Science, 2018).

### 1.3.2 Bioreactors carbon media

Carbon media filling the bioreactors is one of the most important decisions in the design of bioreactors, since it is the substrate for biofilm growth and the carbon source for the microorganisms.

Furthermore, carbon media will affect factors of great importance for denitrification efficiency, such as the hydraulic residence time or the longevity of the bioreactor (Christianson, 2011). Also leached substances such as tannins (which are difficult to degrade by microorganisms and which can color the effluent) are elements to consider. In fact, in the USA it is recommended not to use cedar or other coniferous woodchips (USDA, 2015) because may have antimicrobial or antifungal properties which have not been tested in a woodchip bioreactor (Kjaersgaard, 2013). Other factors as the cost of the substrate, porosity and C: N ratio must be taken into account.

Several works have studied different types of carbon sources for bioreactors, comparing not only lignocellulosic materials of different species but also their size and even the hardness of the woods (Cameron and Schipper, 2010; Addy et al., 2016).

#### 1.3.2.1 Types of carbon media

In the earliest work with denitrification bioreactors, some kind of media as sand, tree bark, compost (Blowes et al., 1994), pine bark, almond, walnut shells, newspaper, cellulose (Volokita et al., 1996; Díaz et al., 2003) were used. In more recent

years, media used have been: sawdust (Warneke et al., 2011b), green waste (Cameron and Schipper, 2010, 2012; Warneke et al., 2011b), corncobs (Christianson et al., 2010), barley straw (Healy et al., 2012), cardboard (Fenton et al., 2014), wheat straw (Saliling et al., 2007; Grießmeier and Gescher, 2018), maize cobs (Warneke et al., 2011a) or woodchips (softwood or hardwood)(Greenan et al., 2006; Robertson, 2010; Gosch et al., 2020).

#### 1.3.2.2 Size of carbon media and hydraulic conductivity

In addition to the type of material, another characteristic to consider is the size of the woodchips. According to studies by Cameron and Schipper (2010), the use of sawdust or woodchips that are too thin (< 6 mm) should be avoided since, in general, they have a much lower hydraulic conductivity than thicker woodchips (between 15 and 60 mm). Hydraulic conductivity is the speed at which the water flow passes through the bioreactor. Apart from depending on the woodchips size, it also depends on the microporosity and the design of the water inlet and outlet of the bioreactor. As a result, if the hydraulic conductivity of woodchips decreases, the life of the bioreactor will too.

Another cause of the decrease in conductivity is the decrease in pore volume as sediment and silt from the inlet water clog the woodchip pores of the bioreactors.

#### 1.3.2.3 Longevity of carbon media

Several are the studies that have studied the evolution of the characteristics of the carbon media throughout the years. Since solid organic carbon media are slowly degraded over time, they provide a long-term source of metabolizable soluble organic carbon. But these duration also depends on type of carbon media, for example straw was the half of useful life as for woodchips according to Grießmeier et al. (2019).

Commonly, woodchips or sawdust are the preferred fill material due to cost, longevity, conductivity, and C:N (Schipper et al., 2010). Many are the types of woodchips studied in denitrifying bioreactor, such as: oak (Schmidt and Clark, 2013), pine (Elgood et al., 2010; Christianson et al., 2011b; Nordström and Herbert, 2018), bamboo, eucalyptus (Forbis-Stokes et al., 2018), poplar, larch, spruce (Bílková et al.,

2018), birch (Kujala et al., 2020), cottonwood (Mardani et al., 2020) and sometimes a woodchips mix are used as Aalto et al. (2020) who used a mix of spruce, poplar and beech.

The quantity and quality of woodchips are important factors in denitrifying bioreactors. The more labile carbon compounds in the woodchips (celluloses and hemicelluloses) break down much earlier than the less labile forms such as lignin, so that over time the proportion of the less labile carbon increases and is more difficult to use by microorganisms for denitrification.

However, despite the woodchips degradation over time, most scientific studies that have assessed their evolution in bioreactors operating at long-term in different parts of the world speak of an average longevity of the woodchips of between 5 and 10 years and even more. Schipper and Vojvodić-Vuković, (2001) studied bioreactors over 5 years with sawdust (Pinus radiata), Moorman et al. (2010) and Christianson et al. (2020) studied bioreactors over a 9 years-period. Long et al. (2011) studied woodchips bioreactors over 14 years (the longest study found). USDA (2020) recommend in the USA bioreactors design lives for more than 10 years.

### 1.3.3 Denitrification influencing factors

#### 1.3.3.1 Kind of water treated and operation regime of bioreactors

Denitrifying bioreactors have been used to treat $NO_3^-$-enriched discharges from a variety of applications, including agricultural tile drainage (Blowes et al., 1994; Christianson et al., 2010, 2014; Feyereisen et al., 2016), wastewater (Dalahmeh et al., 2011), drinking water (Wang and Chu, 2016), stormwater (Lynn et al., 2015; Peterson et al., 2015), aquaculture (Saliling et al., 2007; von Ahnen et al., 2018) or greenhouse effluents.

Inside bioreactors there are two main types of water operating regime. The first type is through a continuous flow, where the water circulates constantly. The second type is in batch mode, with flood cycles, where the bioreactor is flooded, without cover the media, and when time established for the flooding period (hydraulic residence time, a parameter discussed later) is finished, the bioreactors are emptied

until the next flooding cycle. This second type leaves the bioreactors unsaturated for a time, and during that time media is oxygenated, activating aerobic conditions provided breakdown of organic matter and the stimulation of $NO_3^-$ removal during the subsequent flooding cycle (Maxwell et al., 2018).

#### 1.3.3.2 Drying-rewetting cycles

As previously mentioned, periods that carbon media are no saturated can stimulate organic matter breakdown. To know this phenomenon in detail, Maxwell et al. (2018) studied the effects of drying-rewetting cycles in the capacity of bioreactors for $NO_3^-$ removal. This author found an increase in $NO_3^-$ removal rates in woodchip bioreactors when increasing duration of aerobic periods prior to woodchip resaturation (Maxwell et al., 2019).

The hypothesized mechanism for this effect was that drying-rewetting cycles, by briefly exposing the carbon substrate to aerobic conditions, effectively increase carbon availability by promoting aerobic microbial breakdown. Increased degradation of woodchips more frequently exposed to aerobic conditions was seen by Moorman et al. (2010) as greater biomass loss in shallower woodchips, and Ghane et al. (2018) who showed that woodchips closer to a bioreactor inlet, prior to depletion of dissolved oxygen, had greater proportions of recalcitrant carbon as lignin. Aerobic processes are more capable of degrading lignin (Kirk et al., 1987) and yield lower molecular weight carbon molecules (Healy and Young, 1979; Colberg and Young, 1985) that are more bioavailable to denitrifiers. Carbon leaching from organic material decreases quickly (i.e., within a matter of days) upon resaturation after a drying-rewetting cycles (Chow et al., 2006; Hansson et al., 2010; Maxwell et al., 2018) as aerobically-produced carbon is leached or consumed.

#### 1.3.3.3 Hydraulic Residence Time (HRT)

HRT is the contact time between carbon media and the water to be denitrified. Although theoretical HRT is calculated based on the flow through the bioreactor, its volume and substrate porosity, the real HRT varies depending on certain factors, such as water flow distribution between woodchip pores (Christianson, 2011; Christianson et al., 2013).

Monitoring HRT is vitally important in denitrification because it is directly related to denitrification rates, so a higher HRT, greater remove $NO_3^-$ from the water (Greenan et al., 2009; Christianson et al., 2010; Hoover et al., 2016) because more time the denitrifying bacteria have to transform $NO_3^-$ into nitrogen gas.

Therefore, HRT is an important factor in bioreactor design to achieve optimum $NO_3^-$ removal from water. However, excessively long HRT, can reach a total denitrification, this should be avoided to prevent the production of potentially undesirable elements, such as reduced sulfur ($HS^-$, $S^{2-}$ aqueous or $H_2S$ gaseous), which are strong toxins for fish and humans, and methane ($CH_4$), a powerful greenhouse gas and air quality pollutant.

It must also be considered that, as temperature influenced denitrification, HRT will change according to the time of year, requiring greater HRT in winter than in summer to achieve the same efficiency in denitrification.

#### 1.3.3.4 Temperature

Temperature have an important role in denitrification. The relationship between $NO_3^-$ removal rates and temperature are quantified using the $Q_{10}$ temperature coefficient. The $Q_{10}$ coefficient corresponds to the factor by which $NO_3^-$ removal rates increase for every 10 °C increase in temperature, with $Q_{10} = 1$ indicating no temperature effect, and higher $Q_{10}$ values indicating greater sensitivity to temperature.

Reported $Q_{10}$ values for $NO_3^-$ removal in woodchip bioreactors typically range from 1.8 – 4.7 (Elgood et al., 2010; Schmidt and Clark, 2013; Hoover et al., 2016). Unrelated to temperature, $NO_3^-$ removal rates in woodchip bioreactors are also known to generally decrease with time.

The effects of temperature and woodchip age on $NO_3^-$ removal in woodchip bioreactors has generally been determined by quantifying their impact as independent factors. There is evidence, however, of an interaction between the two factors, with temperature effect changing as carbon quality of the woodchip changes over time. Experimental evidence of increased temperature sensitivity of respiration at lower carbon quality has been widely reported (Fierer et al., 2005; Craine et al., 2010;

Wetterstedt et al., 2010). Xu et al. (2012) showed that temperature sensitivity of respiration was inversely correlated with soil organic carbon quality, with higher $Q_{10}$ at lower carbon quality (Xu et al., 2012).

#### 1.3.3.5 pH and Oxidation Reduction Potential (ORP)

The pH and ORP are two key physicochemical parameters influencing and affected by microbial activity of hydric systems (Reddy and DeLaune, 2008; Tercero et al., 2015). The optimum pH range for denitrification is ≈ 5.5 – 8 (Gibert et al., 2008; Rivett et al., 2008; Albina et al., 2019). And this is important because, for example, pine woodchips frequently used in US bioreactors have an excessively low pH for the first few months and denitrification rates are low until most acidic compounds of the substrate are washed.

ORP is an indicator of the activity of both aerobic and anaerobic microorganisms (Fiedler et al., 2007). In well-aerated systems, where microorganisms use free oxygen for their metabolism, ORP values were > ≈ +350 mV (oxic conditions at pH ≈ 7, Vepraskas and Faulker, 2001; Otero and Macias, 2003; Reddy and Delaune, 2008; Unger et al., 2009). In flooded systems, when oxygen concentration falls below ≈ 4 % (ORP ≈ +350 mV), microorganisms use other electron acceptors (e.g., $NO_3^-$) for organic matter mineralization via anaerobic pathways and ORP decreases accordingly. Denitrification occurs at ORP values between ≈ +350 mV and ≈ +100 mV, and sulfate ($SO_4^{2-}$) reduction to sulfide ($S^{2-}$) at ORP values < ≈ +100 mV. So, a drop in ORP in flooded environments is evidence of biological activity, since it reflects oxygen depletion as a consequence of respiration of microbial during organic carbon consumption (Vepraskas and Faulker, 2001; Unger et al., 2009) (Figure 1.5).

### 1.3.4 Bioreactors advantages, drawbacks and managements strategies

Denitrifying bioreactors have several advantages respect to other methods. Are cost-effective, durable, easy to maintain, their design can be tailored to different areas or fields (Schipper et al., 2010), has low external energy requirements (Christianson and Tyndall, 2011) and bioreactors use remains of other activities.

But, also, have several drawbacks, as changes in the characteristics of the water to be treated (temperature, composition, loading) can perturb the biological

metabolism, for example, a decrease in the water temperature decrease water efficiency. In this case, a way to maintain a high denitrification level could be increased the hydraulic residence time.

Also, bioreactors need an external carbon source for microbial activity (Wisniewski et al., 2002), and woodchips, for example, during the start-up of bioreactors could generate a flush of dissolved organic carbon in the effluent (Addy et al., 2016) which can be very harmful to living beings in the water body that receive that effluent. Although it is a temporary problem, a way to reduce the initial carbon flush may be pre-washing the woodchips before the denitrification process begins (Abusallout and Hua, 2017).

Another drawback, if microbial activity is triggered by high dissolved organic carbon concentrations (as occurs during the start-up of bioreactors) and the $NO_3^-$ concentration is not high enough to meet the demand for electron acceptors from microorganisms, sulfate reduction can play a very important role and generate dissolved sulfides and hydrogen sulfide gas.

Other problems could be the emissions of greenhouse gases or organic pollutants (Schipper et al., 2010; Christianson et al., 2011a), for example, the emission of $N_2O$, although its emissions are usually very low concentrations (less than 1% of the input $NO_3^-N$; Warneke et al., 2011a). Also, there could be methane production that occurs when microorganisms use $CO_2$ as an electron acceptor once the sulfate is depleted. This process is difficult to achieve because it is the least energy-efficient and the abundance of $NO_3^-$ and sulfate in the waters to be treated. A way to avoid this situation could be reduced hydraulic residence time, to reduce the dissolved organic carbon inside of the bioreactor (Kinsman-Costello et al., 2015; Li et al., 2017).

In any case, different parameter concentrations must be regularly monitored inside bioreactors and effluents, to avoid the risk they may pose as they are toxic.

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# Chapter 2

# Study Area and background

## 2.1 Site description, background, and motivation

### 2.1.1 Campo de Cartagena

The Campo de Cartagena watershed (1316 $km^2$), Region de Murcia is located in the southeast of Spain (Figure 2.1), is one of the main agricultural fertigation areas of Europe. The zone is characterized by a Mediterranean semiarid climate, with average annual temperatures of 18 ˚C and precipitation of ≈ 300 mm $year^{-1}$, leading to a severe hydric deficit (average potential evapotranspiration of 1275 mm $year^{-1}$) (Jiménez-Martínez et al., 2011). Despite being a dryland area, in the last 40 years agricultural fertigation areas have grown by ten-fold, currently covering about 30 – 38 % of the basin (≈ 40 000 – 50 000 ha) (Álvarez-Rogel et al., 2020). The area is a major supplier to European markets, particularly of horticulture products and citrus.


Figure 2.1. Location maps of the Campo de Cartagena in SE Spain (Council of Europe. European Union, 2015; Google Maps, 2021).

To support the intensive fertigation agriculture in the Campo de Cartagena, there are four main water resources: high quality water supplied by the Tajo-Segura Water Transfer (TSWT), desalinated seawater, effluent from municipal wastewater treatment plants (WWTP) and groundwater withdrawals from the aquifer (Palomar and Losada, 2010; Rico Amorós et al., 2016)(Figure 2.2).

One of the most heavily used water resources among farmers in the region to maintain intensive fertigation has been groundwater withdrawal (Jiménez-Martínez et al., 2016; Díaz-García et al., 2020). However, because the aquifers have become salinized from saltwater intrusion (between 3970 µS $cm^{-1}$ and 6500 µS $cm^{-1}$, Scientific Advisory Group for El Mar Menor, 2017), it is necessary to desalinate before use. In addition, the intense agricultural use led to the nitrate pollution of surface and subsurface waters (Álvarez-Rogel et al., 2020). In fact, $NO_3^-$-N concentration in the Quaternary aquifer ranges from 22 to 34 mg $NO_3^-$-N $L^{-1}$ N (Jiménez-Martínez et al., 2011, 2016), reaching 30 – 45 mg $NO_3^-$-N $L^{-1}$ closer to the coast of the Mar Menor (Tragsatec, 2020).

Desalination process is usually performed in small reverse-osmosis desalination plants installed on local farms. Annually, these plants withdraw ≈100 - 110 $hm^3$ of groundwater and produce ≈20 - 25 $hm^3$ of brine. This high salinity problem is expected to be aggravated according to predictions of the Intergovernmental Panel on Climate Change (Hoegh-Guldberg et al., 2018).

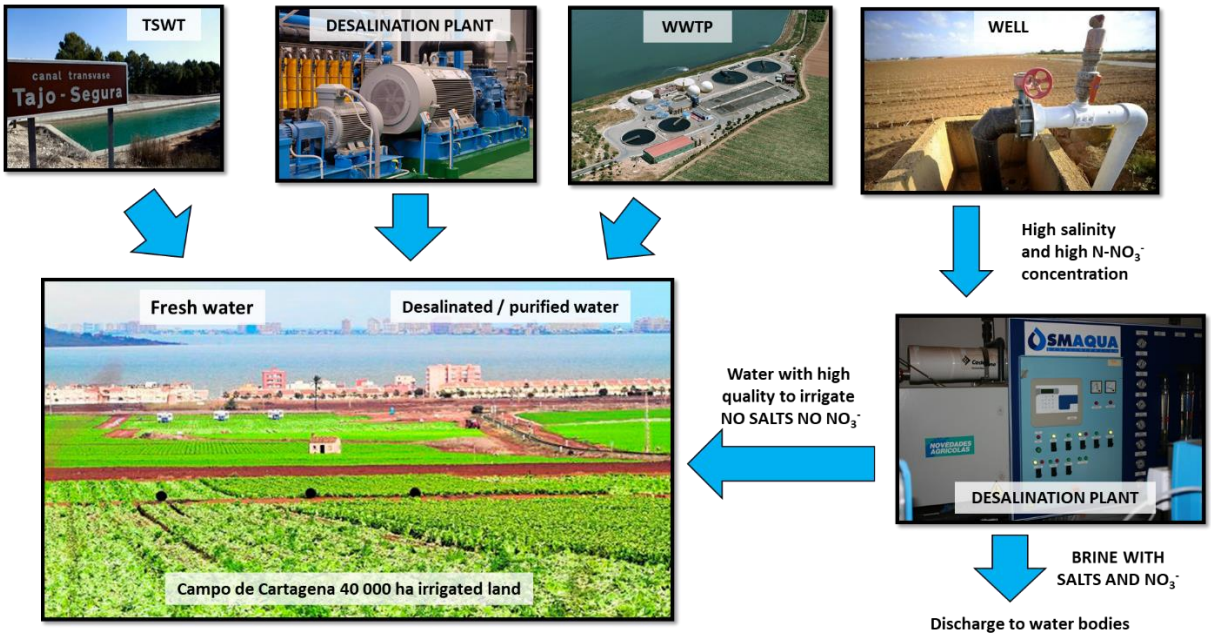


Figure 2.2. Main water resources in the Campo de Cartagena.

Due to high price of desalinated water from the sea desalination plants and the decrease in the supply quota of the Tajo-Segura Water Transfer, farmer do not have enough water to irrigate their fields, so they installed small desalination (Figure 2.3) plants in their farms.


Figure 2.3. Desalination plant.

The desalination plant there are environmental impacts associated with the desalination process since the resulting wastewater from desalination is both brine and contains high concentrations of nitrate. This nitrate-enriched brine has been discharged into the Mar Menor lagoon for many years, contributing to the degradation of the lagoon (Jiménez-Martínez et al., 2016; Scientific Advisory Group for El Mar Menor, 2017; Álvarez-Rogel et al., 2020).

### 2.1.2 The Mar Menor lagoon

The Mar Menor (135 $km^2$, volume ≈ 645 $hm^3$ and mean depth of ≈ 4.5 m) is the largest coastal hypersaline one in the Mediterranean basin. It is separated from the Mediterranean Sea by a narrow sand bar (La Manga del Mar Menor). The Mar Menor is adjacent to the Campo de Cartagena watershed, which surface and subsurface water courses discharge into the lagoon.

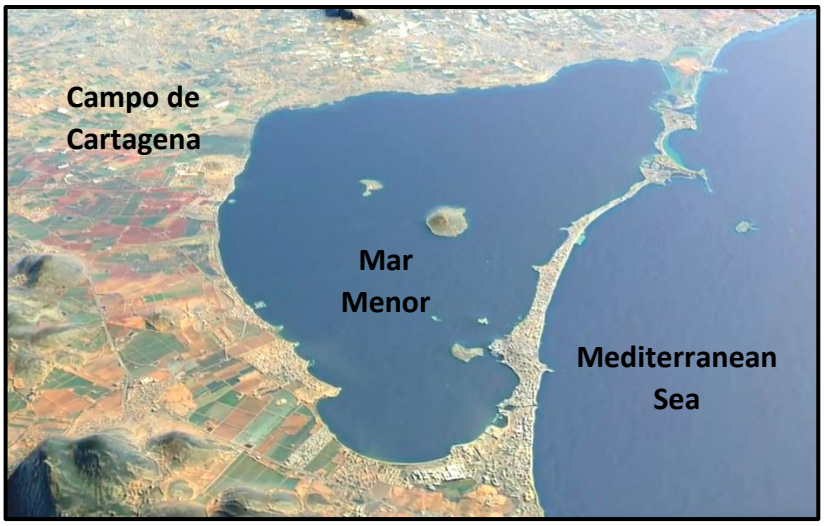


Figure 2.3. Aerial view of Mar Menor lagoon (Calleja, 2017).

This lagoon is included in the Ramsar Convention of wetlands, is a Specially Protected Areas of Mediterranean Importance (SPAMI), Site of Community Importance (SCI) and Special Protection Area (SPA) (Boletín Oficial del Estado (BOE), 2018). As well, the Mar Menor was appointed a Sensitive Area subject to eutrophication in June 2001 under the European Directive 91/721/EEC (Castejón-Porcel et al., 2018). The Mar Menor in conjunction with Campo of Cartagena were declared a Vulnerable Area to Nitrate Contamination under Directive 91/676/EEC. Surface (from watercourses, runoff, and erosion) and subsurface (from the aquifer) nutrient discharges to the lagoon during the last 40 years mainly include effluents and sediments from agricultural fields, occasional discharges from WWTP and sewage network, and brine discharges from desalination plants. The surface watercourses (called ramblas) that only carried water in periods of torrential rains, became a continuous discharge to the Mar Menor with a flow of more than 400 $m^3$ per year and nitrate concentrations exceeding 45 mg $NO_3^-$-N $L^{-1}$ (Álvarez-Rogel et al., 2006; García-Pintado et al., 2007). In addition, according to Ministry for Ecological Transition of Spain (2020) between years 2018 and 2019, ≈360 tons of $NO_3^-$-N were discharged to the Mar Menor by the Quaternary aquifer.

### 2.1.3 Background and motivation

The high nutrient inputs to the Mar Menor changed the lagoon from its original oligotrophic state to a eutrophic state. During some years, the system buffered the eutrophication effects by its self-regulatory mechanisms. However, when the lagoon was pushed beyond its threshold point a phytoplankton bloom was triggered, which peaked in 2016, which turned water turbid and greenish (Ruiz-Fernández et al., 2019). This prevented the light reach the bottom and 85 % of the area covered by benthic macrophytes was killed (Belando Torrente et al., 2019). After that, Regional Government implemented new management regulations to their protection and recovery, including the closure of on-farm desalination plants and the closing of the network of pipes transporting brines to the lagoon. To achieve this purpose they promulgated four legal regulations in three years (Murcia Regional Government, 2017, 2018, 2019, 2020). However, some observations indicated that brines were still being discharged to the lagoon (Álvarez-Rogel et al., 2020).

One of the main regulations, article 13 of Law 1/2018 (Murcia Regional Government, 2018) requires the implementation of a nitrate reduction system in desalination, this implementation remained in the two subsequent laws (Murcia Regional Government, 2019, 2020). So, farmers are required to install a system for denitrification of the nitrate-enriched brine produced during the desalination process to prevent harmful effects of nitrates released to water bodies. The effectiveness of the denitrification system must be previously verified by the Region de Murcia Government by issuing a report of conformity.

As a consequence of this situation, the Cátedra de Agricultura Sostenible para el Campo de Cartagena was created. This Cátedra was created at the Universidad Politécnica de Cartagena in March 2017 as an initiative of 13 agricultural cooperatives from Campo de Cartagena, the Federación de Cooperativas Agrarias de Murcia (FECOAM) and for the Coordinadora de Organizaciones de Agricultores y Ganaderos - Iniciativa Rural de Murcia (Coag-Ir). Lately the company INSAL and Fundación Obra Social La Caixa were added. The purpose was to promote research and innovation in the agricultural sustainability area as well as to encourage theoretical and practical training actions aimed at professionals in the agricultural sector.

One of the main objectives of the Cátedra was the start-up of a pilot project focused on implementing denitrification systems with woodchip bioreactors in the Campo de Cartagena. The main aim was to explore options for reducing the nitrate load of rejection brine from desalination plants. For that, a pilot plant was built in the Agri-food Experimental Station Tomás Ferro (Estación Experimental Agroalimentaria Tomás Ferro-ESEA) from the UPCT to carry out experimental trials.

In addition, based on the Universidad Politécnica de Cartagena desalination plant and the Cátedra progress, an Operational Group called “Innovación en calidad del agua de riego y la sostenibilidad ambiental (AGUAINNOVA)” was requested made up of the Coordinadora de Organizaciones de Agricultores y Ganaderos del Campo de Cartagena (COAGACART), (FECOAM) and COAG-IR MURCIA whose objective was to contribute to the research carried out by Cátedra.

In 2018, ESAMUR (Regional Entity for Sanitation and Wastewater Treatment of the Region de Murcia) sponsored the R&D contract “Design and monitoring of a pilot plant with wetlands and bioreactors for the treatment of agricultural drainage water in Campo de Cartagena” ("Diseño y seguimiento de una planta piloto con humedades para el tratamiento de las aguas de drenaje agrícola del Campo de Cartagena") managed by Universidad de Murcia and Universidad Politécnica de Cartagena. For that, the company built a pilot plant in WWTP Los Alcázares, in order to study wetlands and denitrifying bioreactors in the treatment of water from D7 drainage ditch, one of the main channels collecting agricultural effluents in the Campo de Cartagena.

This thesis includes the main findings obtained in both pilot plants between 2016 and 2020. In the ESEA plant woodchips bioreactors worked in a batch mode to denitrify brine from a desalination plant, and in Los Alcázares plant woodchips bioreactors worked under continuous flow to denitrify effluents flowing in the D7 ditch.

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# Chapter 3

## Objectives

## 3.1 Objectives

Considering the numerous worldwide evidence that have proven the effectiveness of woodchip bioreactors for denitrifying a variety of nitrate enriched effluents, the initial hypothesis of this thesis was that these systems can be an effective tool to mitigate the problem of nitrate contamination described in the Campo de Cartagena. According to this hypothesis, the main objective of this thesis was to evaluate whether the use of bioreactors to denitrify brines and other saline agricultural effluents with a high nitrate load is feasible in the Campo de Cartagena (Southeast of Spain). Only few works have studied the efficacy of denitrifying bioreactors for the treatment of saline water highly enriched in nitrate. Particularly, there are lack of studies denitrifying brine from desalination plants in woodchip bioreactors. Hence, this thesis can be considered a novel contribution to the state of the art of denitrifying bioreactors research.

The specific objectives were:

1. To assess the viability of almond shell, chopped carob, olive bone and citrus woodchips as carbon media for denitrifying brine with high nitrate load, by considering nitrate removal efficiency and efficiency:cost ratio.
2. To perform a comprehensive evaluation (including effects of seasonal temperature, changes in dissolved organic carbon release, and woodchip age) of the behavior and efficiency of woodchip bioreactors to denitrify brine from a groundwater desalination plant.
3. To perform a comprehensive analysis looking at the effect of the interaction between carbon quality and temperature on nitrate removal rates in woodchips. The analysis used woodchip age and time elapsed since a DRW cycle as indicators of carbon quality/availability.
4. To assess the efficiency of citrus woodchips bioreactors working under continuous flow regime for reducing the high $NO_3^-$ loads from agricultural leachates flowing in surface water courses of the Campo de Cartagena.
5. To get a preliminary assessment of how different hydraulic residence times affect woodchips degradation and assess the existence of potentially harmful substances in the bioreactor effluents.

# Chapter 4

# Selection of a suitable carbon media to use in bioreactors

The content of this chapter has been published in the following scientific article:

Díaz-García, C., J.J. Martínez-Sánchez, and J. Álvarez-Rogel. 2020. Bioreactors for brine denitrification produced during polluted groundwater desalination in fertigation areas of SE Spain: batch assays for substrate selection. Environ. Sci. Pollut. Res. (2): 1–10. doi: https://doi.org/10.1007/s11356-020-09567-6.

# Bioreactors for brine denitrification produced during polluted groundwater desalination in fertigation areas of SE Spain: batch assays for substrate selection

## 4.1 Introduction

Although bioreactors have been used for denitrification of brackish water (e.g., aquaculture with salinities from 0 to 35 ppt and ≈ 22.6 mg $NO_3^-$-N $L^{-1}$; (von Ahnen et al., 2019)), they have never been used for the denitrification of concentrated brine with high nitrate load (EC ≈ 20 mS $cm^{-1}$ and ≈ 67 - 80 mg $NO_3^-$-N $L^{-1}$). This would be a novel application of denitrifying bioreactors that can contribute to reduce the environmental impacts of brine. To the authors' knowledge, this is the first work of denitrifying brine using organic wastes as the carbon substrate, and the first use of these carbon media for that purpose. It is necessary to verify the potential of bioreactors in the treatment of saline water before promoting their use among farmers for denitrification of brine from reverse-osmosis.

In this study, four locally-available carbon media were tested as candidates for carbon media in denitrifying bioreactors: almond shell, chopped carob, olive bone and citrus woodchip (Table 4.1). The four carbon media were selected for being relatively cheap, easy to obtain, and largely accessible in Mediterranean coastal areas of south Europe (Figures 4.1 to 4.4).

Table 4.1. Summary of all carbon medias, assays and HRT used in the experiment.

| | Citrus woodchip | Almond shell | Chopped carob | Olive bone |
|---|---|---|---|---|
| **First assay** | 1, 12 and 36 h | | | |
| **Second assay** | 1, 2, 4, 6, 8 and 10 h | | | |
| **Third assay** | 2, 4, 6 and 10 h | | | |

Figure 4.1. Olive bones using in the assays.

Figure 4.2. Almond shell using in the assays.

Figure 4.3. Chopped carob using in the assays.

Figure 4.4. Citrus woodchips using in the assays.

Almond shells account for about 20 % of the total weight of almond production and Spain produces 51 thousand tons of almond per year, or 33 % of the 2.2 million tons of world production (Martínez Gutiérrez et al., 2009). Chopped carob consists of pieces of carob fruit, a crop with 2600 tons annual production in Spain and 1.4 million tons production globally; it is traditionally used for animal feed with almost 90 % carbohydrates, 50 % of which are sugars. Olive bone comprises 15 % of the olive fruit and is composed of cellulose, hemicellulose and lignin (Alami, 2010), with annual Spanish olive production of 6.5 million tons (FAO, 2017). Citrus woodchips are available from cultivated citrus trees, with more than 290 000 hectares of citrus production in Spain (Statista, 2017).

This study aimed to get a preliminary assessment of the viability of almond shell, chopped carob, olive bone and citrus woodchip as carbon media for denitrification of brine in bioreactors by considering nitrate removal efficiency as well the efficiency:cost ratio. Brine effluents from bioreactors batch assays containing the

four carbon media were analyzed for several physical and chemical parameters, dissolved organic carbon and nitrogen release, nitrate removal, and a cost comparison of each carbon media was provided. Although laboratory small scale experiments such as this are only a first step for future medium and large-scale field applications, they can provide important insights for testing new carbon media (e.g., Peterson et al. (2015); Malá et al. (2017)).

## 4.2. Materials and methods

### 4.2.1 Carbon media and brine characterization

Carbon media were characterized for bulk density, porosity, pH, electrical conductivity (EC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and $NO_3^-$-N. Containers of known volume were filled with each carbon media and weighed to calculate bulk density as the mass:volume ratio. Bulk density was measured using methods similar to Christianson et al. (2010). Distilled water was added over 2 h to dried media, filling the drainable porosity and internal pore space of the carbon media; the total volume added was considered the effective porosity of a bioreactor filled with the corresponding waste. The pH values were measured with portable devices by inserting pH electrodes (Crison 50–50) two hours after the initial saturation for the media. Samples were then collected, filtered (Albet 145 filter, 7 – 11 µm pore diameter) and analyzed for EC, DOC, TDN and $NO_3^-$-N.

Brine was obtained from a reverse osmosis desalinization plant installed at the Agri-food Experimental Station Tomás Ferro (ESEA) by School of Agricultural Engineering of Polytechnical University of Cartagena (ETSIA - UPCT). The plant desalinizes water withdrawn from the Quaternary aquifer of Campo de Cartagena. Brine was analyzed for EC, pH and $NO_3^-$-N, and had characteristics of: EC = 19 mS $cm^{-1}$ (≈12 g $L^{-1}$ salt); pH = 7.55; $NO_3^-$-N = 79 mg $NO_3^-$-N $L^{-1}$. These parameters are in the typical range of values seen in brine produced by local desalination plants in the region of interest, with high salinity, pH slightly above neutral, and a $NO_3^-$-N concentration between 67 and 90 mg $NO_3^-$-N $L^{-1}$.

### 4.2.2. Bioreactors design and operation

Three successive batch assays were performed in 20 L capacity plastic containers (n = 3 for each carbon media: almond shell, chopped carob, olive bone and citrus woodchip; Figure 4.5) according to a completely randomized factorial design. In each assay, the containers were filled with brine and a protocol of sample collection and physical-chemical monitoring was followed as described below.

Figure 4.5. Batch bioreactors used in the assays.

The first of the three assays lasted for 36 hours. The oxidation-reduction potential (ORP) and pH were measured at 0.5, 24 and 36 hours after initial saturation. Effluent brine samples (25 mL) were collected after 6 and 36 hours of flooding for DOC as well as TDN analysis. After 36 hours the containers were emptied then immediately refilled with new brine, beginning the second of the three assays. The second assay lasted a total of 10 hours. ORP, pH and temperature were measured at 0.5, 2, 4, 6, 8 and 10 hours after saturation of the carbon media. Effluent brine samples (25 mL) were collected after 0.5, 6 and 10 hours of flooding for measurement of DOC, TDN and $NO_3^-$-N. At the end of the second assay, the containers were emptied and refilled a second time with new brine, beginning the final and third of the three assays. The third assay lasted for 10 hours and, based on preliminary results obtained from the first two assays, was only performed with citrus woodchip as carbon media. ORP, pH and temperature were measured (Figure 4.6) then samples collected at 0.5, 2, 4, 6 and 10 hours after saturating the media and analyzed for DOC, TDN, and $NO_3^-$-N.


Figure 4.6. Detailed view of the thermometer and pH/Eh electrodes used in the batch bioreactors assays.

ORP and pH in all the experiments were measured with portable devices by inserting Crison 50 - 55 and 50 - 50 electrodes, respectively. Values for ORP were adjusted according to Vepraskas and Faulker (2001), by adding +200 mV to the measured voltage of the Ag/AgCl reference electrode at 20 °C. Temperature was measured with an electronic digital thermometer WT-1.

### 4.2.3. Analytical methods

Measurements of EC were made with a Crison Basic 30. DOC and TDN were analyzed with a LECO series 628 analyzer at support service for technological research of UPCT (SAIT); likewise $NO_3^-$-N with a V/UV spectrometer at λ = 220nm, with interference by organic matter corrected by measuring the absorbance at λ = 275 nm (AOAC, 1975).

Concentrations of dissolved organic nitrogen (DON) in the samples collected from bioreactors were estimated by subtracting $NO_3^-$-N from TDN. We assume that this method of calculating DON led to a certain degree of error since other forms of inorganic N (e.g., $NH_4^+$-N, $NO_2^-$-N) were not considered. However, since $NO_3^-$-N brine (≈ 80 mg $NO_3^-$-N $L^{-1}$) and organic-N released by the carbon media were so high we assumed that our approach was suitable to obtain estimation about the use of carbon media for microorganisms.

### 4.2.4. Statistical analyses

Statistical analyses were performed with IBM SPSS Statistic 22 (significant differences at $p < 0.05$). Data were log-transformed when necessary to achieve homogeneity of variances (Levene's test). For each parameter, repeated measures ANOVA (RM-ANOVA) were applied to dependent variables (pH, ORP, DOC, $NO_3^-$-N) of the treatments (e.g., carbon media) over time. In each experiment, a significant effect of time indicated that changes of the dependent variable (e.g., pH) were significant with increased duration of flooding, regardless of the carbon media. A significant effect of the time x treatment interaction term indicated that changes of the dependent variable over time were different for different carbon media. Finally, a significant effect of treatment indicated that the measured values of a dependent variable were significantly different among carbon media. For determining the significance of treatment effect, Tukey post-hoc tests were performed to identify differences between carbon media for the specific water quality parameter.

## 4.3. Results

### 4.3.1 Characteristics carbon media

The lowest bulk density values (≈ 250 kg $m^{-3}$) besides the highest porosity values (≈ 56 %) were found in citrus woodchip (Table 4.2). Chopped carob had lower bulk density than olive bone, but both had similar porosity. Regarding salinity, olive bone showed the highest EC (≈ 11 mS $cm^{-1}$) followed by the chopped carob (≈ 4.7 mS $cm^{-1}$), which were both higher than EC for almond shell and citrus woodchip (Table 4.2). All the carbon media showed lightly acidic to acidic pH, with the highest average value in citrus woodchip (≈ 5.7) and the lowest in chopped carob (≈ 4.7). As expected, there were high concentrations of DOC and TDN in the assay discharge water, particularly in the chopped carob (≈ 790 mg TDN $L^{-1}$). The four carbon media leached $NO_3^-$-N with the largest concentration in chopped carob (≈ 4.38 mg $NO_3^-$-N $L^{-1}$) and the lowest in citrus woodchip (≈ 2 mg $NO_3^-$-N $L^{-1}$).

Table 4.2. Characteristics of the carbon media used in the different assays. EC: electric conductivity; DOC: dissolved organic carbon; TDN: dissolved total nitrogen; $NO_3^-$-N: nitrate-nitrogen; $NO_2^-$-N: nitrite-nitrogen; $NH_4^+$-N: ammonium-nitrogen; % Org-N: Percentage of organic-nitrogen; % Inor-N: Percentage of inorganic-nitrogen. The values are the mean ± standard error (n = 3). Parameters measured after flooding the carbon media with distilled water for two hours.

| | **Almond shell** | **Chopped carob** | **Olive bone** | **Citrus woodchip** |
|---|---|---|---|---|
| **Bulk density (kg $m^{-3}$)** | 296 ± 21.36 | 420 ± 4.59 | 726* | 250 ± 8.40 |
| **Porosity (% volume)** | 55.6 ± 1.1 | 47.8 ± 0.4 | 34.7 ± 2.75 | 56.6 ± 1.6 |
| **EC (mS $cm^{-1}$)** | 1.26 ± 0.19 | 4.78 ± 0.09 | 11.54* | 2.60 ± 0.25 |
| **pH** | 5.37 ± 0.07 | 4.69 ± 0.01 | 5.3* | 5.69 ± 0.01 |
| **DOC (mg $L^{-1}$)** | 1735 ± 461 | 92093 ± 11036 | 5059 ± 359 | 2112 ± 421 |
| **TDN (mg $L^{-1}$)** | 46.79 ± 2.82 | 789 ± 60 | 255 ± 15 | 201 ± 44 |
| **$NO_3^-$-N (mg $L^{-1}$)** | 1.81 ± 0.01 | 4.38 ± 0.73 | 1.65 ± 0.9 | 2.04 ± 0.07 |
| **$NO_2^-$-N (mg $L^{-1}$)** | 0.43 ± 0.001 | 0.31 ± 0.08 | n.d | 0.34 ± 0.21 |
| **$NH_4^+$-N (mg $L^{-1}$)** | 0.31 ± 0 | 16.66 ± 2.5 | n.d | 8.20 ± 1.2 |
| **% Org-N** | 94.54 ± 0.3 | 97.31 ± 0.3 | n.d | 94.63 ± 1.1 |
| **% Inor-N** | 5.46 ±0.3 | 2.69 ± 0.3 | n.d | 5.37 ± 1.1 |

* no replicates were available for this treatment. 10 mg $kg^{-1}$ is the N detection limit.

n.d – no data available

### 4.3.2 Evolution of physical-chemical properties, DOC and nitrogen following flooding with brine in the four carbon media

In the first and second assays, pH, ORP, DOC and TDN were affected by time ($p \leq 0.001$) and the interaction term of time x treatment ($p \leq 0.009$), indicating that concentrations of the parameters changed significantly during flooding but at different rates according to carbon media. In the first assay, citrus woodchip always maintained a significantly higher pH (between 5.6 and 6.2) throughout the 36 hours of flooding, relative to the other media, while pH was lower in the other three carbon media, between ≈ 4.4 and ≈ 5.2, with smaller differences between these three media (Figure 4.7A).

However, at the beginning of the second assay, after emptying and refilling the containers, pH in citrus woodchip increased ≈ 1.5-fold, almond shell ≈ 2-fold and olive bone ≈ 1-fold. By contrast, chopped carob had lower pH values than in the first assay. The pH values were significantly different among the four carbon media at all sampling times during the second assay (Figure 4.7B).

Similar ORP values were obtained for almond shell, citrus woodchip and olive bone at 0.5 hours after saturation during the first assay (between ≈ +270 and +350 mV), with chopped carob having significantly lower ORP (≈ +180 mV) than the other media (Figure 4.7C). During the experiment, ORP was fairly constant in chopped carob (≈ +200 mV) and almond shell (≈ +350 mV) but decreased to < 0 mV in the other two carbon media. After 36 hours of flooding, the four carbon media had significantly different ORPs, with the lowest observed value (≈ -200 mV) in citrus woodchip.

After refilling the bioreactors again during the second assay (Figure 4.7D), ORP decreased to ≈ +85 mV in almond shell and increased to ≈ +70 mV in olive bone during the first hour, and did not change significantly ($p > 0.05$) throughout the 10 hours of flooding. In chopped carob ORP was similar to values seen in the first assay (≈ +140 to +200 mV) and significantly higher than in the other three carbon media. Finally, the ORP in citrus woodchip was always lower than 0 mV, reaching the minimum value (≈ -220 mV) after 10 hours of flooding.

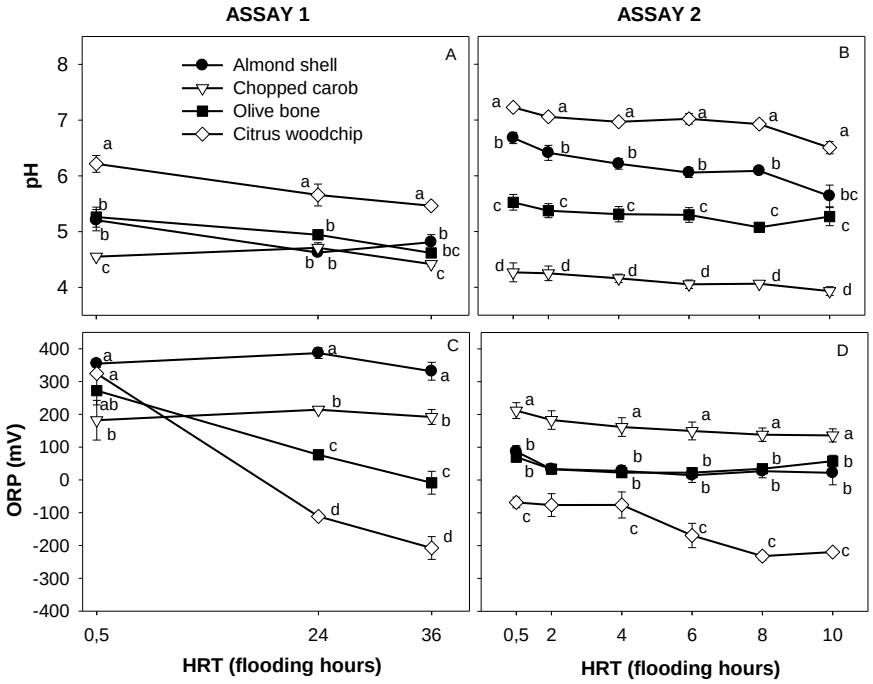


Figure 4.7. Evolution of pH (A and B) and ORP (C and D) during the first and second assay with brine. Values are the mean ± standard error (n = 3 for each treatment). Different letters for a sampling time indicate significant differences among treatments at $p < 0.05$ (RM-ANOVA and Tukey post-hoc test). HRT: Hydraulic Retention Time.

DOC tended to decrease between 6 and 36 hours of hydraulic retention time (HRT) during the first assay in the four carbon media (Figure 4.8A). Olive bone as well as citrus woodchip had the lowest DOC concentrations, being relatively similar at 6 and 36 h without significant differences between them at both sampling times (≈ 2000 mg C $L^{-1}$ at 6 h and ≈ 500 mg C $L^{-1}$ at 36 h). Chopped carob showed the highest DOC concentrations (≈ 35 000 mg C $L^{-1}$ at 6 h and ≈ 20 000 mg C $L^{-1}$ at 36 h). Concentrations of DOC in almond shell were intermediate (≈ 3900 mg C $L^{-1}$ at 6 h and ≈ 3700 mg C $L^{-1}$ at 36 h) (Figure 4.8A).

At the beginning of the second assay (Figure 4.8B), DOC concentrations decreased in almond shell (≈ 6 to 10 - fold) and citrus woodchip (≈ 2 - fold) relative to the end of the first assay but increased in olive bone (≈ 3 - fold), while DOC concentrations in chopped carob were similar to the first assay. Contrasting with results from the first assay, almond shell showed lower DOC than olive bone. DOC tended to increase in all of the carbon media over 10 hours of saturation during the second assay.

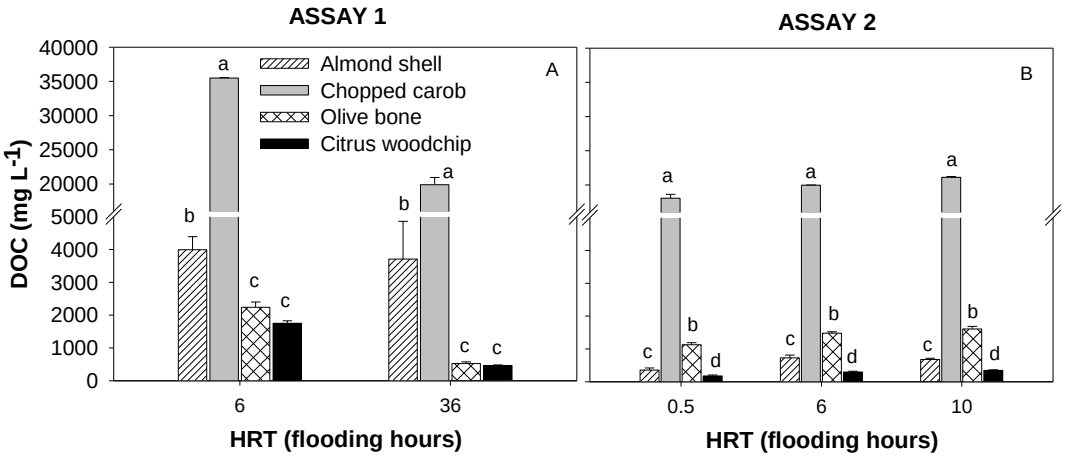


Figure 4.8. Concentrations of DOC during the first (A) and second (B) assay with brine. Values are the mean, with error bars indicating the standard error (n = 3 for each treatment). For each sampling time, different letters above bars indicate significant differences among treatments at $p < 0.05$ (RM-ANOVA and Tukey post-hoc test). HRT: Hydraulic Retention Time.

TDN and $NO_3^-$-N concentrations in the second assay were significantly higher in chopped carob and generally increased during the 10 hours of saturation (from ≈600 to ≈800 mg N $L^{-1}$ and from ≈ 400 to ≈ 590 mg N $L^{-1}$, Figure 4.9A and 4.9B). The other three carbon media had similar TDN as well as $NO_3^-$-N concentrations after 0.5 hour of saturation. TDN was significantly lower in almond shell than in olive bone at 6 and 10 hours, but $NO_3^-$-N only decreased in the latter carbon media after 10 hours of flooding. However, in citrus woodchip, TDN as well as $NO_3^-$-N were significantly lower than in the other carbon media at 6 h and 10 h (≈ 30 - 40 and ≈ 14 - 18 mg $L^{-1}$ respectively). The only carbon media in which $NO_3^-$-N was consistently reduced after 6h of flooding was in citrus woodchip (Figure 4.9).

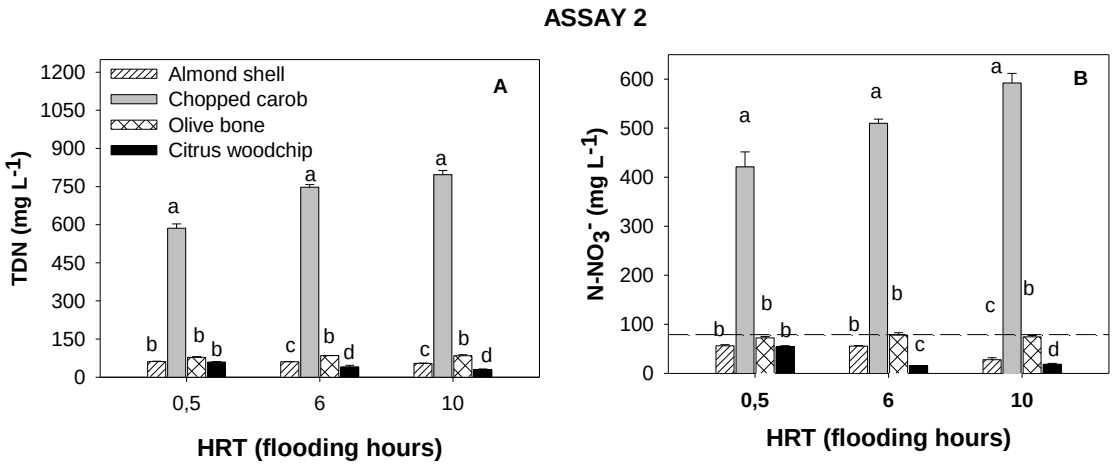


Figure 4.9. Concentrations of TDN (A), $NO_3^-$-N (B) during the second assay with brine. Values are the mean with error bars showing the standard error. n = 3. For each sampling time, different letters above bars indicate significant differences among treatments at $p < 0.05$ (RM-ANOVA and Tukey post-hoc test). Dashed line in B indicates $NO_3^-$-N concentration in brine. HRT: Hydraulic Retention Time.

### 4.3.3 Changes in pH, ORP, DOC, TDN, DON and $NO_3^-$-N in woodchips bioreactors during the third assay

At the beginning of the third assay, where only woodchips were used as carbon media due to their better performance, pH showed similar values seen at the end of the second assay. This parameter was relatively stable at pH ≈ 7.45 during the 10 hours of HRT of the third assay (Figure 4.10A). However, ORP significantly decreased starting at ≈ -4 mV one hour after flooding and reaching ≈ -202 mV after 10 hours (Figure 4.10A). Concentrations of $NO_3^-$-N decreased strongly by 12 - fold, from ≈ 67 mg $NO_3^-$-N $L^{-1}$ to ≈ 5 mg $NO_3^-$-N $L^{-1}$ (Figure 4.10B), and DOC increased 4 - fold, from ≈ 40 mg C $L^{-1}$ to ≈ 160 mg C $L^{-1}$ (Figure 4.10C). As a consequence of the increase in soluble organic matter (as shown by DOC) and the large decrease of $NO_3^-$-N, organic N, a minor fraction of the N species present at the beginning of the experiment, was the main N species after 10 hours of flooding (Figure 4.10D).

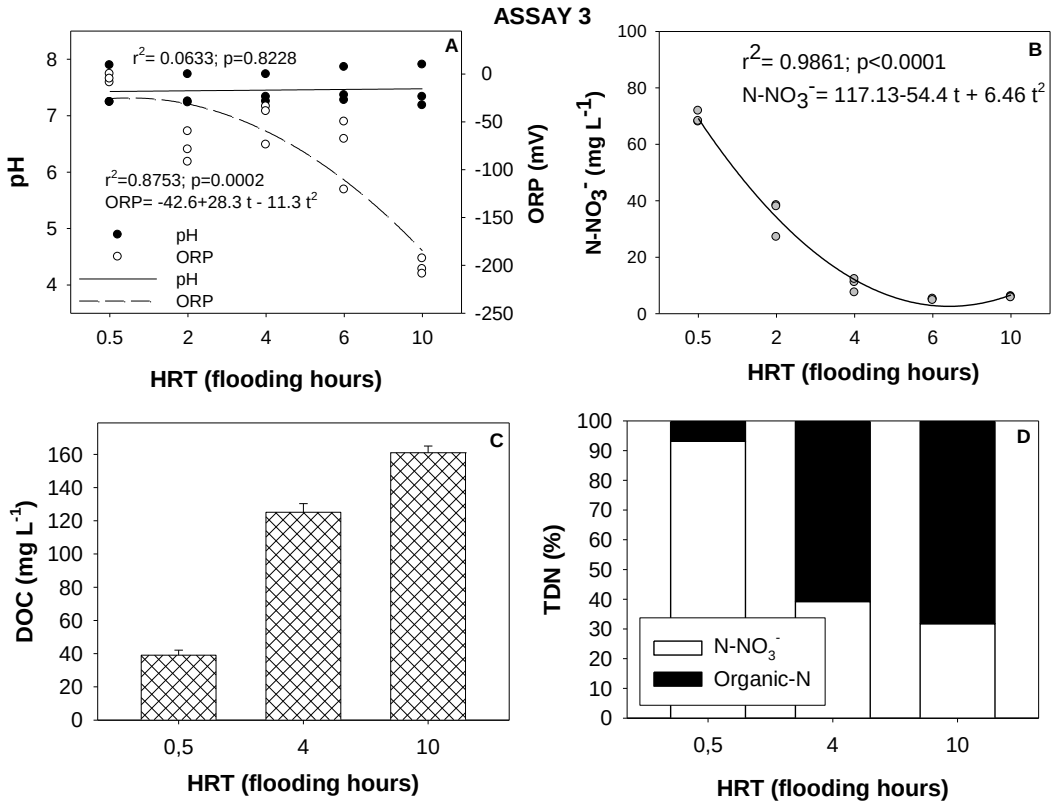


Figure 4.10. Evolution of pH, ORP, DOC, TDN and $NO_3^-$-N during the third assay with brine. During the third assay only citrus woodchips were analyzed. Values in the bar plots show the mean with error bars showing the standard error (n=3). HRT: Hydraulic Retention Time.

## 4.4. Discussion

The low pH values at the beginning of the first assay (Figure 4.7A) can be related to the high concentrations of acidic organic compounds that were solubilized when carbon media were mixed with distilled water (von Ahnen et al., 2019), as indicated by the high concentrations of DOC (Figure 4.8A). It is important to consider that pH is one of the main parameters influencing activity of denitrifying bacteria, with an optimum range of pH = 5.5 – 8.0 (Gibert et al., 2008). Hence, values pH < 5.5 during the first and second assays in chopped carob and olive bone could hamper microbial activity, while in almond shell this only happened in the first assay. In the citrus woodchip treatment, the pH values were always > 5.5, within the range optimum microbial activity.

Salinity is another factor that influences the activity of microorganisms. Although denitrification occurs in non-saline as well in saline environments (Reddy and DeLaune, 2008; Álvarez-Rogel et al., 2016), high salinity can affect the process by preventing microorganisms from maintaining their osmotic pressure balance, giving rise to bacterial plasmolysis (Lay et al., 2010). Zhao et al. (2013) found a reduction in denitrification rate at 20 g $L^{-1}$ NaCl concentrations, and Von Ahnen et al. (2019) revealed that salinity altered the woodchip microbiome, promoting autotrophic denitrifiers and decreasing the overall denitrification potential.

We did not directly evaluate microbial activity, but ORP is a parameter that reflects the relative activity of both aerobic and anaerobic microorganisms (Fiedler et al., 2007). A drop in ORP in flooded environments is evidence of biological activity, since it reflects oxygen depletion as a consequence of respiration of microbial during organic carbon consumption (Vepraskas and Faulker, 2001; Unger et al., 2009). The data showed decreases in ORP, but the variations were different for separate media and assays, likely due to the differences in pH and salinity and the C and N composition of the carbon media. The anoxic conditions (ORP < ≈ +350 mV; Otero and Macias, 2003) reached in olive bone and citrus woodchip during the first assay (Figure 4.7C) can be related to the larger DOC decrease in these treatments (Figure 4.8A). The recalcitrant composition of almond shell (hollocellulose 64.3 %, α cellulose 29.1 %, lignin 32.7 % and ash 3.4 %; Pirayesh and Khazaeian, 2012), in combination with the acidity of this media (pH≈5), could hinder microbial activity, as shown by the relatively high absolute values and small decreases in ORP values (≈ +350 mV, Figure 4.7C) and DOC (≈ 4 000 mg $L^{-1}$, Figure 4.8A) at 6 and 36 hours of flooding. Chopped carob had an intermediate behavior, showing a decrease of DOC as well as suboxic conditions (+100 ≤ ORP ≤ +350 mV; Otero and Macias, 2003) probably because of its high concentration of sugars (Biner et al., 2007) which stimulated microbial activity and oxygen consumption leading to a decrease in ORP. Chopped carob produced a high level of TDN relative to the three other carbon media, which can be explained by their composition; chopped carob has a TDN content of 1.26 %, (Sciammaro, 2015), compared to a TDN content of 0.48%, 0.21% and 0.1 % for olive bone (Alami, 2010),

almond shell (Ingaman Dos S.L, 2014), and citrus woodchips (García Garrido, 2011), respectively.

After emptying brine from the bioreactors and subsequently refilling (second assay), the observed pH increases indicate that most of the soluble acidic organic compounds were leached when bioreactors were drained at the end of the first assay (Figure 4.8B). However, this was strongly dependent on the type of carbon media, as shown by the significant differences in pH values (Figure 4.7B) and DOC concentrations (Figure 4.8B) during the second assay.

Through the second assay, ORP values were similar to values seen at the end of the first assay, except for almond shell in which ORP decreased to anoxic conditions which was not the case in the first assay. The higher pH reached in almond shells could explain this, with higher microbial activity and oxygen consumption at higher pH leading to a drop in ORP values. In fact, almond shell was able to reduce $NO_3^-$-N concentration after 10 hours of flooding (≈ 64 % efficiency), while olive bone and chopped carob did not see substantial reductions. The largest $NO_3^-$-N removal was found in citrus woodchip bioreactors, reaching ≈ 76 % efficiency.

Nitrate removal efficiency increased at longer retention times during the third assay with woodchip, from 12.3 % after 1 h to 92.6 % after 10 h of HRT. In terms of Specific Nitrate-Reduction Rate (SNR) the efficiency was 9.7 ± 2.1 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ to 1 h and 3.6 ± 0.01 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ to 10 h of HRT. These values showed similar trends to that of Christianson et al. (2012) which used yard waste as the carbon source. At 6 hours of HRT, nitrate removal efficiency was 93.2 %, which corresponded to 6.1 ± 0.06 mg $NO_3^-$-N $m^{-3}$ $d^{-1}$ of SNR, showing that 6 hours were enough to achieve a high level of removal efficiency.

One concern is the increase in concentrations of COS and organic N in the brine after being in contact with the carbon media. The latter is a common feature at the beginning of bioreactor operation (Healy et al., 2012; Malá et al., 2017), but the concentrations strongly decrease after the first weeks when this excess pool of potential pollutants is washed away and the system reaches steady-state operation conditions (Fenton et al., 2014; Malá et al., 2017). Since denitrifying bioreactors can have a usable lifetime of up to 10 years (Schipper et al., 2010; Fenton et al., 2014), the

initial release of organic compounds is not considered a drawback, although it would need to be collected and properly managed in the early phase of operation, particularly if limits on organics in brine discharges exist.

Since future field applications of bioreactors in farms are linked not only with denitrification capacity but also with economic concerns, cost estimations of the carbon media are a key aspect. Based on the current market, volumetric costs of the four carbon media (per cubic meter) are 19 € for almond shell, 96 € for chopped carob, 36 € for olive bone, and 5.76 € for citrus woodchip. According to these estimates, citrus woodchip was not only the most effect media for nitrate reduction but is also economically the most favorable media.

## 4.5. Conclusions

Our approach is the first attempt for assessing the use of almond shell, chopped carob, olive bone and citrus woodchip as carbon media for denitrification of brine in bioreactors. Our findings indicated that denitrifying bioreactors are a suitable option for denitrification of brine. Citrus woodchip were the most favorable carbon media, since it showed the lowest leaching of organic carbon and nitrogen, the highest reductions of nitrate, and had the lowest cost of any of the media selected. Chopped carob and olive bone provided negligible reductions in nitrate in the brine; chopped carob generated a highly acidic leachate with extremely high dissolved organic carbon, while olive bone produced a highly saline leachate. Almond shell, one of the most abundant carbon media in the Mediterranean area, was effective for denitrification, but its high cost and recalcitrant organic carbon would strongly limit its usefulness. Next phases of this research include field scale pilot experiments to evaluate the medium and long-term performance of citrus woodchips for the denitrification of nitrate-rich brine.

## 4.6. Acknowledgements

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# Chapter 5

# Woodchip bioreactors for treating brine from groundwater desalination plants

The content of this chapter is under review to be published:

Díaz-García, C., J.J. Martínez-Sánchez, B.M. Maxwell, J.A. Franco, and J. Álvarez-Rogel. Woodchip bioreactors provide sustained denitrification of brine from groundwater desalination plants. Journal of Environmental Management. Under review.

# Woodchip bioreactors provide sustained denitrification of brine from groundwater desalination plants

## 5.1. Introduction

Preliminary trials showed that citrus woodchips are a suitable organic substrate for brine denitrification (Díaz-García et al., 2020), however longer assays are necessary prior the regional adoption of this technology to better understand the influence of factors such as temperature changes, hydraulic residence time and woodchip age on nitrate removal performance.

This study covers the results of a comprehensive 2.5-year pilot-scale study for applying citrus woodchips bioreactors to denitrify nitrate-enriched brine from desalination of groundwater in the Campo de Cartagena agricultural watershed. We aim to evaluate the processes implied in bioreactor performance and nitrate removal efficiency. The effects of seasonal temperature changes dissolved organic carbon release, hydraulic residence time, and woodchip age in bioreactor performance are discussed. To the authors' knowledge, this is the first study to look at the efficacy of woodchip bioreactors for the treatment of brine from desalination. Hence, our results can be considered a novel contribution to the state of the art in woodchips bioreactors research.

## 5.2. Materials and methods

### 5.2.1 Description of the experimental set up

A 121-week pilot scale experiment was conducted at the Agri-food Experimental Station Tomás Ferro (ESEA) (N 37° 41' 17.6" and W 0° 57' 04.4") of the School of Agricultural Engineering, Technical University of Cartagena (ETSIA-UPCT), Cartagena, Region of Murcia, Spain, between November 2017 and March 2020. The pilot plant is equipped with a well (depth = 50 m) for groundwater withdrawal (Table 5.1), a desalination plant with reverse osmosis (capacity of 80 $m^3$ $d^{-1}$) which produces 68.7 % fresh water and 31.3 % brine by volume and a number of bioreactors (Figure 5.1).

Figure 5.1. Citrus woodchips bioreactors in the pilot plant at the Agri-food Experimental Station Tomás Ferro (ESEA) of the School of Agricultural Engineering, Technical University of Cartagena (ETSIA - UPCT) to denitrification experiment with brines.

Brine was stored on-site in an opaque tank for 48 h at ambient temperature to ensure continuous availability for the batch experiments. Woodchip bioreactors used for the experiment described in the present paper consisted of three rectangular, above-ground fiberglass containers (142 x 108.5 x 85 cm). Each bioreactor contained a vertical PVC pipe (63 mm of diameter) placed within the woodchip media with holes at 25 cm from the bottom of the bioreactor to allow water to enter the well (Figure 5.2).

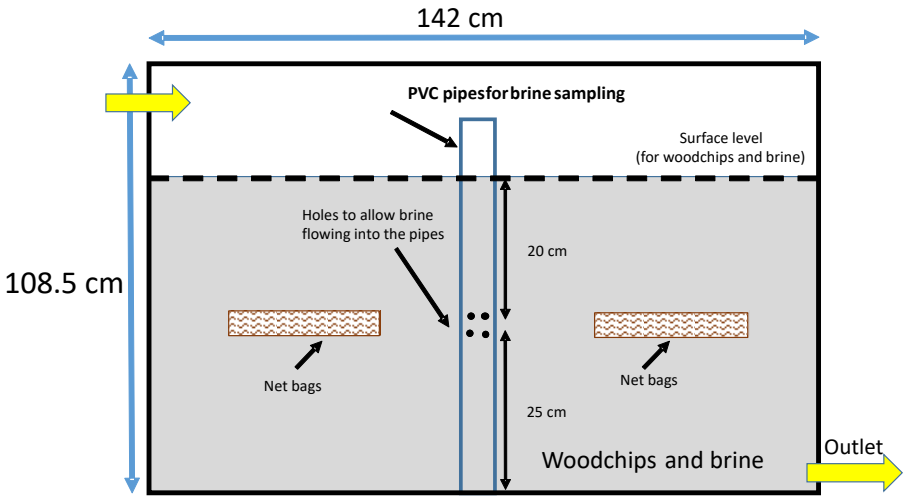


Figure 5.2. Scheme of a woodchip bioreactor using for brine denitrification.

Each bioreactor was filled with 122 kg of citrus woodchips (Table 5.2) which are readily available in Mediterranean coastal areas of south Europe. Two net bags (30 x 60 cm with 2 x 2 mm sized mesh), each with 1 kg of dried (at 65 °C) woodchips, were placed at 25 cm from the bottom in each bioreactor to assess weight loss of woodchips over the duration of the experiment.

Table 5.1. Characteristics of groundwater extracted from wells at the study site and the brine used in the batch experiments. Values are the mean ± standard error. Average, minimum (Min.) and maximum (Max.) were reported based on samples from the entire study period (121 weeks). Groundwater well samples, n = 48; brine samples, n = 253. EC: electrical conductivity; ORP: oxidation-reduction potential.

| | **Well water** | | **Brine** | |
|---|---|---|---|---|
| **Parameters** | **Average** | **Min. - Max.** | **Average** | **Min. - Max.** |
| **pH** | 7.33 ± 0.03 | 6.88 – 7.71 | 7.77 ± 0.02 | 5.65 – 8.23 |
| **ORP (mV)** | 244 ± 8.7 | 140.1 – 381.2 | 231.1 ± 3.8 | 119.6 – 403.1 |
| **EC (mS cm$^{-1}$)** | 6.2 ± 0.1 | 5.2 – 7.4 | 17.7 ± 0.1 | 16 - 20 |
| **$NO_3^-$-N (mg L$^{-1}$)** | 18.1 ± 0.2 | 14.9 – 24.8 | 48.5 ± 0.3 | 38.6 – 59.3 |
| **$Cl^-$ (mg L$^{-1}$)** | 1636 ± 15 | 1400 - 1847 | 5007 ± 33 | 3907 – 6967 |
| **$SO_4^{2-}$ (mg L$^{-1}$)** | 1440 ± 12 | 1265 - 1641 | 4543 ± 34 | 2587 - 6658 |
| **$Ca^{2+}$ (mg L$^{-1}$)** | 341 ± 2.5 | 303.4 – 383.5 | 1066 ± 5.1 | 793 - 1262 |
| **$Mg^{2+}$ (mg L$^{-1}$)** | 268.6 ± 2.1 | 236.7 - 307 | 858 ± 5.3 | 682 - 1229 |
| **$Na^+$ (mg L$^{-1}$)** | 969.4 ± 7.8 | 844.8 - 1083 | 3019 ± 22 | 2340 - 4147 |

Table 5.2. Characteristics of the citrus woodchips used in the experiment. Average length of the woodchips; Average diameter of the woodchips; Bulk density; Porosity; EC: electrical conductivity; pH; DOC: dissolved organic carbon; $NO_3^-$-N: nitrate-nitrogen. The values are the mean ± standard error (n = 3). Parameters measured after flooding citrus woodchips with distilled water for two hours.

| **Parameter** | **Data** | **Parameter** | **Data** |
|---|---|---|---|
| **Average length (mm)** | 35.7 ± 1.7 | **EC (mS cm$^{-1}$)** | 2.60 ± 0.25 |
| **Average diameter (mm)** | 5.19 ± 0.4 | **pH** | 5.69 ± 0.01 |
| **Bulk density (kg m$^{-3}$)** | 230.9 ± 7.7 | **DOC (mg L$^{-1}$)** | 2112 ± 421 |
| **Porosity (% volume)** | 56.6 ± 1.6 | **$NO_3^-$ -N (mg L$^{-1}$)** | 2.04 ± 0.07 |

### 5.2.2 Experimental design, monitoring and sampling

Woodchip bioreactors were operated in batch mode with three batch runs performed each week at a 24 h hydraulic residence time (HRT). Each Monday at ≈ 8 a.m. each bioreactor was filled with brine until water level in the bioreactor was even with the surface of the woodchip media (average 242.2 ± 1.3 L of brine per bioreactor). Woodchips remained fully saturated for a 24 h period (until Tuesday 8 a.m.), after which tanks were completely drained. After removing the brine, bioreactors were immediately refilled (< 1 h after draining) and woodchips resaturated with new brine for the next 24 h batch experiment. On Wednesday 8 a.m. they were emptied and refilled again for a third 24 h batch. On Thursday 8 a.m. of each week they were emptied for the third time, after which the woodchip media remained unsaturated without brine for 96 hours until starting the next 24 h batch on Monday of the following week. This mode of operation was designed based on expected operational guidelines for farmers in the Campo de Cartagena. In total, 765 batches were performed. A total of 186 $m^3$ of brine were denitrified during the experiment. Denitrified brine was stored in a detention basin for further management and off-site disposal.

From weeks 1 to 96, bioreactors were monitored and sampled during each of the three weekly batches. From week 97 onwards, bioreactors were monitored and sampled only during the second weekly batch (on Tuesday), although three batches were still performed each week. Samples of groundwater were collected from the well every two weeks (total samples 48). Samples of brine were collected each day prior to filling the bioreactors (total samples 253) (Table 5.1). After saturating the woodchips, brine pH, temperature, electrical conductivity (EC) and oxidation-reduction potential (ORP) were measured by inserting a calibrated multiparameter instrument (Hanna HI 98194 pH/EC/DO Multiparameter) within the vertical PVC sampling well. These measurements were made at 30 min, 10 h and 24 h of HRT. Values for ORP were adjusted according to Vepraskas and Faulker (2001), by adding +200 mV to the measured values (the voltage of the Ag/AgCl reference electrode at 20 °C). During sampling of the water in the bioreactors, brine within the vertical PVC sampling pipes was first vacated using a polyethylene (PE) sampler, allowing the pipe to refill with

brine in contact with the woodchips, prior to taking *in situ* measurements and collecting samples. Brine samples were collected at 10 h HRT by extracting water from the vertical PVC pipes using the PE sampler, and at 24 h HRT from the outlet of the bioreactors. Samples were filtered through Microsart CN-Filter filters (0.45 μm pore size). Additionally, one of the woodchip-filled net bags was removed from each bioreactor at 12 and 24 months after the experiment began. Bags were oven dried at 65 °C until constant weight and weighed to assess woodchip loss.

### 5.2.3 Analyses

*Woodchip characterization.* Bulk density and porosity of the woodchips were measured according to Christianson (2010). For bulk density, 2 L containers were filled with woodchips and weighed to calculate the mass:volume ratio. Then, distilled water was added over 2 h filling the drainable porosity and internal pore space of the woodchip, where total volume of distilled water added was considered the effective porosity. Aliquots of the distilled water were collected after the 2 h and analyzed for EC, pH, dissolved organic carbon (DOC) (carbon analyzer TOC-V CSH Shimadzu), and $NO_3^-$-N (double channel chromatographic system 850 Professional Ion Chromatography y Metrohm).

*Well and brine sample analyzes*. Samples of well water and brine were analyzed for $NO_3^-$, $Cl^-$, $SO_4^{2-}$, $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, with a double channel chromatographic system 850 Professional Ion Chromatography Metrohm. Samples from each of the three weekly batches were analyzed during first 53 weeks (first 12 months), while samples from the second batch (Tuesday) each week were analyzed during the rest of the experiment, with 1312 samples measured in total. DOC concentration was only measured for samples collected from the second batch of each week (Tuesday) throughout the experiment (583 samples were analyzed; carbon analyzer TOC-V CSH Shimadzu). All water chemistry analysis was performed at the Technological Research Support Service (SAIT) of the Technical University of Cartagena.

In order to evaluate the performance of the bioreactors, Nitrate Removal Efficiency (NRE) and $NO_3^-$-N Removal Rates ($R_{NO3}$) were calculated for each batch run according to Christianson et al. (2015). (Eq. (1 and 2)):

$$\text{NRE}\ (\%) = \frac{(\text{NO}_3^- \text{–N influent concentration} - \text{NO}_3^- \text{–N effluent concentration})}{\text{NO}_3^- \text{–N influent concentration}}\ x\ 100 \quad (1)$$

$$\text{R}_{\text{NO}_3}(\text{g N m}^{-3}\ \text{d}^{-1}) = \frac{(\ \text{NO}_3^- \text{–N influent concentration} - \text{NO}_3^- \text{–N effluent concentration})}{V_{saturated\ woddchips}\ \ x\ t} \quad (2)$$

Where influent concentration was the $NO_3^-$-N in the initial brine of each batch (g N $m^{-3}$), effluent concentration was the $NO_3^-$-N in the effluent after 24 hours (g N $m^{-3}$), $V_{saturated\ woodchips}$ was the volume of saturated woodchips ($m^3$) and t was the time of the nitrate measurement (d).

Measured values of $R_{NO3}$ and water temperature inside the PVC well were used to calculate $Q_{10}$, whose values were used as a metric for temperature sensitivity of nitrate removal $Q_{10}$ is defined as the factor by which a rate of reaction increases for each 10 °C increase in temperature. Data were fitted to equation (3) and equation (4) to calculate $Q_{10}$ values. Collected data was fitted to the relationship in equation (3) using the nls() function in R Studio (RStudio, 2020) a function finding the least-squares parameter estimates of a nonlinear function, solving for R and k (Maxwell et al., 2020a).

$$R_T = R_0\ xe^{kT} \quad (3)$$

$$Q_{10} = e^{10\ x\ k} \quad (4)$$

## 5.3. Results

### 5.3.1 Physicochemical conditions (ORP and pH)

Between weeks 1 to 53 (≈ 1st year, middle Fall 2017-middle Fall 2018) the variability in ORP between the three bioreactors was higher than from 54th week onwards (middle Fall 2018 - Winter 2020), mainly at 30 min and 10 h after saturation, as shown by the higher standard error values during the first period (Figure 5.3). Additionally, ORP values and their seasonal variations differed between the two periods mentioned.

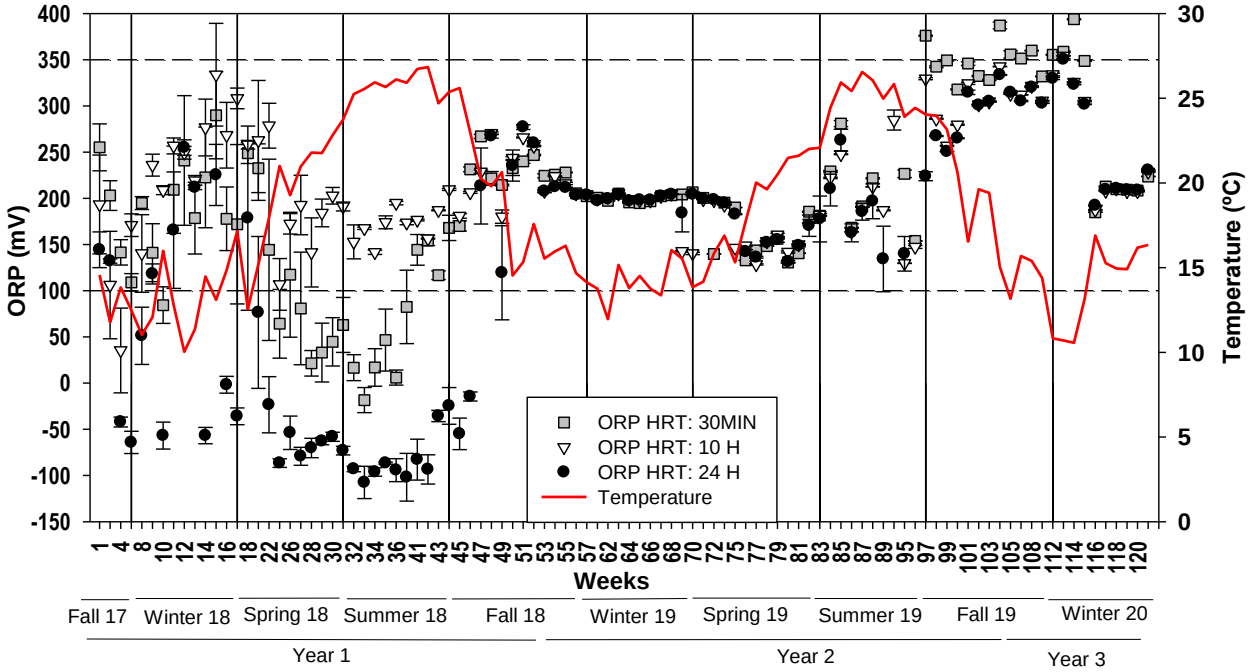


Figure 5.3. Weekly average of Oxidation Reduction Potential (ORP) at 30 min, 10 h and 24 h HRT, and temperature, inside the bioreactors, for the 121 weeks of the experiment. Values are the mean ± standard error. Weeks from 1 to 96, n = 9 (three sampling days with three repetitions per day); weeks from 97 to 121, n = 3 (one sampling day with three repetitions). HRT: Hydraulic Residence Time. Dashed lines show the range of suboxic conditions considered optimal for denitrification (ORP between +100 and +350 mV) (Otero and Macias, 2003).

During the first year, the ORP at 30 min HRT fluctuated from ≈ +150 to ≈ +350 mV when average temperatures was ≈ 10 °C (weeks ≈ 1 to ≈ 18 and ≈ 43 to ≈ 53) (Figure 5.3). When temperature increased (weeks ≈ 18 to ≈ 41) ORP values decreased reaching ≈ 0 mV. ORP at 10 h HRT also tended to decrease at warmer temperatures, although the drop was less pronounced than at 30 min HRT (ORP was always between ≈ +150 to ≈ +350 mV). Finally, during the first year ORP at 24 h HRT was almost always between ≈ -100 and 0 mV, regardless of temperature.

ORP behavior was different during the second year of operation, relative to the first year (Figure 5.3). Between ≈ 53 and ≈ 83 weeks (temperature ≈ 14 - ≈ 17 °C), ORP was fairly stable and similar among the three HRT (≈ +150 to ≈ +200 mV), but when temperature increased (> ≈ 25°C) some distinction was observed (weeks ≈ 83 to ≈ 97) although values were always between ≈ +150 and ≈ +250 mV. During ≈ 97 to ≈ 114 weeks the ORP increased up to ≈ +350 - ≈ +400 mV at 30 min HRT and up to ≈ +300 mV

at 10 h and 24 h HRT. Finally, from week ≈ 115 onwards the ORP values at the three HRT were similar (≈ +20 mV).

In contrast to ORP, pH inside the bioreactors appeared relatively constant during the 121 weeks of the experiment, with an average pH of 7.60 ± 0.007 at all HRT (n = 1689; data not shown). EC values were also stable over the experiment and during batches, with a mean value of 17.74 ± 0.05 mS $cm^{-1}$ (n = 1452; data not shown).

### 5.3.2 DOC and $NO_3^-$-N concentrations

On the first batch run of the first week of the study period (1 - 1 in Figure 5.4), DOC concentrations (Figure 5.4) reached ≈ 1567 mg $L^{-1}$ at 10 h HRT. By the third batch run of the first week (1 - 3 in Figure 5.4), DOC concentrations had dropped to ≈ 300 mg C $L^{-1}$ and decreased further to ≈ 10 mg C $L^{-1}$ between weeks ≈ 8 to ≈ 17 (winter 2018). From weeks 18 to 43 (spring and summer 2018), when temperatures rose, DOC increased again to ≈ 15 - 20 mg C $L^{-1}$ at 10 h HRT and ≈ 30 - 40 mg C $L^{-1}$ at 24 h HRT. Between weeks ≈ 43 and ≈ 108 (second year, fall 2018 – fall 2019) DOC concentrations were ≈ 10 - 16 mg C $L^{-1}$, regardless of HRT and temperature, and from week ≈ 109 (winter third year) until the end of the experiment DOC was relatively stable (≈ 7 mg C $L^{-1}$).

Weight loss (average ± SE) of woodchips contained inside the net bags, relative to the initial weight, was higher during the first year (31.3 ± 0.9 %) than during the second year (10.9 ± 0.2 %).

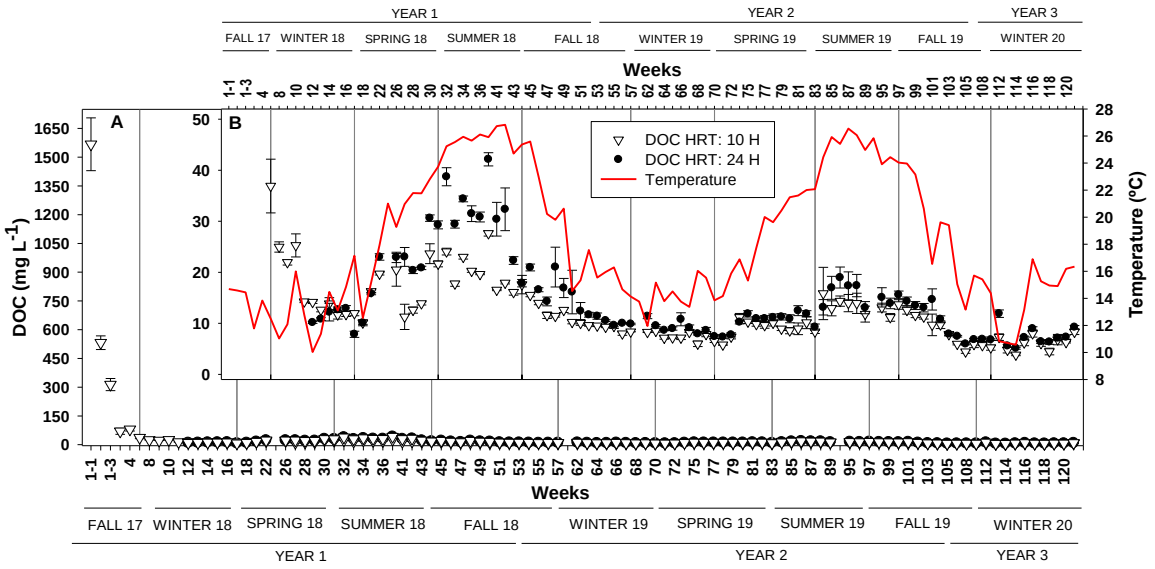


Figure 5.4. Weekly average of Dissolved Organic Carbon (DOC) at 10 h and 24 h HRT, and temperature, inside the bioreactors, for the 121 weeks of the experiment (Figures 5.4A and 5.4B are the same figure with a different range in the Y axe). Values are the mean ± standard error. Weeks from 1 to 96, n = 9 (three sampling days with three repetitions per day); weeks from 97 to 121, n = 3 (one sampling day with three repetitions). HRT: Hydraulic Residence Time. During the first week data are shown as a daily basis (from the first day, 1 - 1, to the third day, 1 - 3).

$NO_3^-$-N concentrations in the inflow were between 40 and 50 mg $NO_3^-$-N $L^{-1}$ throughout the 121 weeks of the experiment (Figure 5.5A). During the first ≈ 17 weeks $NO_3^-$-N in the effluent was ≈ 10 - 30 mg $NO_3^-$-N $L^{-1}$ at 10 h HRT (NRE ≈40 - 90 %, Figure 5.5B) and < ≈ 10 mg $L^{-1}$ at 24 h HRT (NRE ≈ 80 - 95 %, Figure 5.5B), but between weeks ≈ 18 - 45 it was < ≈ 6 mg $NO_3^-$-N $L^{-1}$ at both HRT (NRE > ≈ 80%). During weeks ≈ 49 to ≈ 75, effluent $NO_3^-$-N concentrations increased to ≈ 20 - 30 mg $NO_3^-$-N $L^{-1}$ at 10 h (NRE ≈ 35 - 50 %) and 24 h (NRE ≈ 50 - 70 %), coinciding with temperature decreased, and between weeks ≈ 75 - ≈ 89 decreased again at ≈ 13 mg $NO_3^-$-N $L^{-1}$ (10 h HRT, NRE ≈ 65 %) and ≈ 3 mg $NO_3^-$-N $L^{-1}$ (24 h HRT, NRE ≈ 95 %). After week 89, $NO_3^-$-N gradually increased to 35 to 45 mg $NO_3^-$-N $L^{-1}$ at both HRT (NRE ≈ 25 - 40 %), when temperatures dropped to ≈ 11 - 16 °C. Nitrate Removal Rate ($R_{NO3}$) was similar at both HRT throughout the 121 weeks of experiment (Figure 5.5B) and ranged from ≈ 15 to 25 g N $m^{-3}$ $d^{-1}$ between weeks 1 to ≈ 104 (fall 2017-middle fall 2019), and from ≈ 5 to 10 g $m^{-3}$ $d^{-1}$ between 105 to 121 weeks (middle fall 2019-winter 2020).

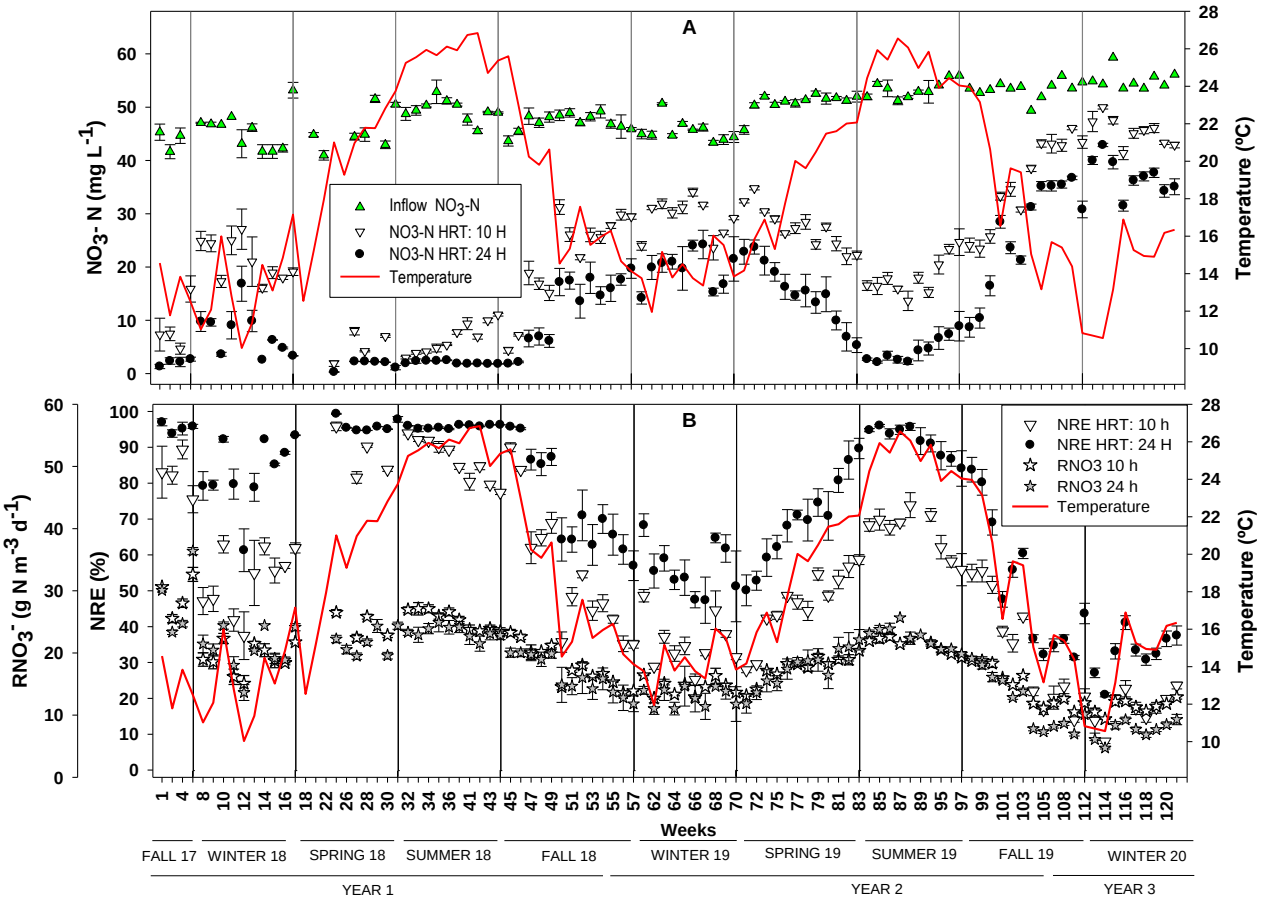


Figure 5.5. A) Weekly average of nitrate ($NO_3^-$-N) concentration in the inflow and in the effluent at 10 h and 24 h HRT, and temperature inside the bioreactors. B) Weekly average of Nitrate Removal Rates ($R_{NO3}$) and Nitrate Removal Efficiency (NRE), and temperature, in the bioreactors. Values are the mean ± standard error. Weeks from 1 to 96, n = 9; weeks from 97 to 121, n = 3. HRT: Hydraulic Residence Time.

## 5.4. Discussion

### 5.4.1 Variations in pH, ORP, EC and DOC with respect to temperature and woodchip age

The pH and ORP are two key physicochemical parameters influencing and affected by microbial activity of hydric systems (Reddy and DeLaune, 2008; Tercero et al., 2015).

In the bioreactors, the pH was relatively stable with low variability throughout the 121 weeks (average of ≈ 7.6 (SE = 0.007)), falling within the range of pH that is known to be suitable for denitrification (pH ≈5.5 – 8) (Rivett et al., 2008; Albina et al., 2019). In flooded systems, an increase in pH is usually expected as ORP decreases due to $H^+$ consumption (Stumm and Sulzberger, 1992). Moreover, denitrification produces alkalinity, which often increases pH (Reddy and DeLaune, 2008). However, in this experiment a slight decrease of pH (average ≈ 0.5) was observed in the effluent at 24 h HRT relative to the initial brine, as also observed by Robertson and Merkley (2009) and Warneke et al. (2011). This decrease could be due to several factors such as the dynamics of the $CO_2$-$H_2CO_3$ system and N nitrification during drying phases (Reddy and DeLaune, 2008; Tercero et al., 2015). The $CO_2$ produced during mineralization of the carbon could have dissolved in the water and formed $H_2CO_3$, a weak acid that contributed to the drop of pH observed. Nitrification, which releases $H^+$, could occur when $O_2$ entered in the bioreactors during drying periods. Furthermore, the organic acids released from the woodchips during flooding could also contribute to the decrease of pH (Albina et al., 2019).

Although the microbial activity was not directly evaluated in this work, ORP is an indicator of the activity of both aerobic and anaerobic microorganisms (Fiedler et al., 2007). In well-aerated systems, where microorganisms use free oxygen for their metabolism, ORP values were > ≈ +350 mV (oxic conditions at pH ≈ 7, (Vepraskas and Faulker, 2001; Otero and Macias, 2003; Reddy and DeLaune, 2008). In flooded systems, when oxygen concentration falls below ≈ 4 % (ORP ≈ +350 mV), microorganisms use other electron acceptors (e.g., nitrate) for organic matter mineralization via anaerobic pathways and ORP decreases accordingly. The cited authors indicated that, at pH ≈ 7, denitrification occurs at ORP values between ≈ +350 mV and ≈ +100 mV, and sulfate ($SO_4^{2-}$) reduction to sulfide ($S^{2-}$) at ORP values < ≈ +100 mV. Since $SO_4^{2-}$ content in the brine was high (≈ 4475 mg $L^{-1}$), the ORP values between +100 and -100 mV measured at 30 min and 24 h HRT during the first 41 weeks indicate potential environmental risks due to sulphate reduction.

Dissolved $S^{2-}$ is highly toxic for biota (Reddy and DeLaune, 2008), and would be an issue if bioreactor effluents are discharged into natural water bodies. It may be necessary to regularly monitor bioreactors treating brine with high $SO_4^{2-}$ concentrations and manage the HRT to avoid ORP conditions leading to formation of these compounds.

Reduced sulfur in bioreactor effluents could be managed using a complementary system with capacity to remove $S^{2-}$, such as a constructed wetland (Vymazal, 2014). Furthermore, a combination of both systems has been shown to have additional advantages for improving the performance and resilience of water treatment under shock loading events of other key contaminants such as TSS, BOD5 and TN (Sukias et al., 2018).

During the first ≈ 24 - 26 weeks (until mid-spring 2018) there was high variability in ORP values at 30 min and 10 h HRT. This could have been due to the start-up period of the bioreactors, where physical, biogeochemical or microbiological properties in the woodchip media had not yet stabilized. Porosity was variable as woodchips were settling, woodchips were possibly less uniform in their nutrient content, and microbial community not fully established. Low temperatures in week ≈ 30, which ranged from ≈ 10 °C to ≈ 15 °C, may have also contributed to the variability found. In a mesocosm study mimicking eutrophic wetlands, Tercero et al. (2015) found that at this temperature range microbial activity was disadvantaged and more irregular than at higher temperatures.

Temperature had an apparent large effect on the behavior of water chemistry in the bioreactors as the experiment progressed and become a decisive factor from spring 2018 onwards (as discussed later), when ORP and DOC began to rise or fall in relation to warming or cooling (Figures 5.3 and 5.4). Between weeks ≈ 30 and ≈ 48, as temperature rose to > ≈ 15 °C and DOC concentrations increased, both factors likely contributed to greater microbial activity, both aerobic and anaerobic, and was reflected by a lower ORP over this period. During this same period ORP values at 30 min HRT were lower than at 10 h HRT. This may seem contradictory if we expect that in flooded systems $O_2$ is progressively depleted as a consequence of microorganism's activity. If so, the longer flooding time, the less oxygen content is expected.

However, the results obtained may be explained as follows. If at the beginning of each batch some anoxic brine from the previous batch remained in the pores of the woodchips, this previously denitrified brine could cause the sharp drop in ORP observed at 30 min HRT. When the conditions of the new brine (which introduced $O_2$ and $NO_3^-$, two oxidants) were prevalent, the ORP would have increased and stabilized to a certain level, as reflected by the values obtained at 10 h (≈ +150 to +200 mV). Later, $O_2$ consumption by microorganisms led to ORP drop again until reaching values indicative of anoxic conditions (< +100 mV) at 24 h HRT. From week ≈ 49 onwards ORP variability decreased, which suggests that the system was physically (e.g., pore spaces) and microbiologically (e.g., microorganisms' population) more homogeneous.

Another important factor for bioreactor performance is the longevity of woodchips (Moorman et al., 2010). Weight loss of the woodchips was higher in the first year than in the second (≈ 31 % and ≈ 11 %, respectively). During the first year of bioreactor operation, with fresh woodchips, microorganisms had greater access to labile carbon (high cellulose content). As the woodchips progressively aged, the quantity and quality of DOC would have decreased, with the woodchips becoming more recalcitrant and therefore more difficult for its rapid consumption by microorganisms (Masbough et al., 2005; Maxwell et al., 2020b). The weight losses found in this work were higher than those reported by Schipper and Vojvodić-Vuković (2001) and Moorman et al. (2010) after 5 and 9 years respectively.

The flow regime is other key factor in woodchips degradation. In the previously cited studies, the bioreactors were operated under continuous flow, while those in the current study were done in batch mode. Woodchips in these batch experiments also remained unsaturated for a period of four days empty (from Thursday to Monday). These phases of drying and rewetting have been shown to promote greater degradation of woodchips via aerobic breakdown since aerobic decomposition is normally more efficient than anaerobic (Bridgham et al., 1998; Moorman et al., 2010; Maxwell et al., 2018). For that reason, denitrifiers would have had greater access to more labile carbon immediately following unsaturated periods that made lower molecular weight carbon more available via aerobic processes (Maxwell et al., 2020a).

These drying-rewetting cycles also increase carbon leaching, with DOC content being higher at the beginning of the flooding phases but decreasing quickly (i.e., within a matter of days) upon resaturation as aerobically-produced carbon is leached or consumed by microbes as a result of the more rapid aerobic degradation (Chow et al., 2006; Hansson et al., 2010; Maxwell et al., 2018). This gradual leaching/loss of labile carbon was reflected in our experiment by the progressive decrease of NRE from Monday (just after four days of bioreactors drying) to Wednesday (the third consecutive weekly flooding batch) (Figure 5.6). The loss of more labile carbon over time would also explain the downward trend of NRE and $R_{NO3}$ over the 121 weeks experiment. DOC production from woodchips could occur at irregular pulses inside bioreactors and not in a homogeneous way, until those woodchips of different shapes and sizes were settled, and pore space conditions were homogenizing. Moreover, quantity and quality of DOC (an issue discussed below) could be more variable during the first months when woodchips were more heterogeneous, and some pieces could be more prone to provide easily metabolizable carbon than others.

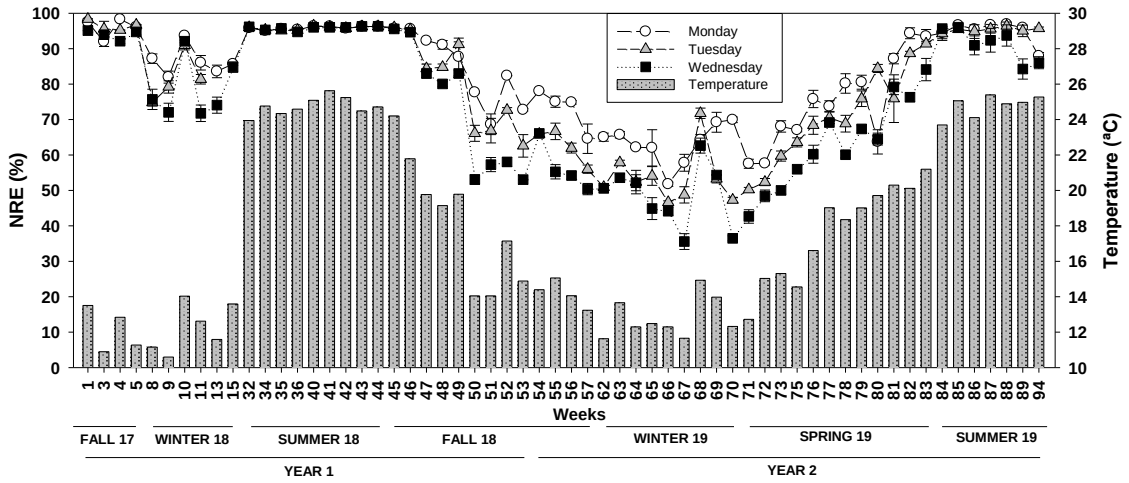


Figure 5.6. Daily average of Nitrate Removal Efficiency (NRE) in the effluents at 24 h HRT of Monday, Tuesday and Wednesday and temperature inside the bioreactors. Values are the mean ± standard error. Weeks from 1 to 94 (n = 3). HRT: Hydraulic Residence Time.

Previous research has shown that high salinity can increase organic matter breakdown and decomposition rates (Craft, 2007; Weston et al., 2011; Marton et al., 2012). For instance, Steele and Aitkenhead-Peterson (2013) showed that organic carbon leaching from senesced vegetation remains increased with sodicity due to the interaction of sodium ions with organic functional groups that increase their solubility. In addition, the high salinity of brine could lead to strong osmotic potential gradient between internal pores of woodchips and the macropore water, could have much contribute to DOC accumulation, with initial diffusion being a major driver. In fact, other experiments at the UPCT facility showed DOC in the effluent of woodchips was greater as brine became more concentrated (Maxwell et al., 2020a).

The decrease in quality/quantity of DOC over time may explain differences in how nitrate removal responded to temperature changes. Contrasting with the warm period (Tª > ≈ 20 °C) of 2018 (weeks ≈ 18 to 44), when temperature increased in 2019 (weeks ≈ 79 to 100) the ORP did not decrease lower than +100 mV, possibly due to the lower quality (more recalcitrance) of the DOC available that hindered microbial activity in some way. Later, the drop in temperature between ≈ 100 and ≈ 112 weeks (fall 19-early winter 2020) combined with the low DOC concentrations (< 10 mg $L^{-1}$) may have been the cause of lower N removal rates and the observed rise in ORP. Robertson (2010) and Maxwell et al. (2020a) indicated that the influence of temperature on microbial activity become more important in aged woodchips and attributed this behavior to the worse media quality together with the more difficult for microorganisms to work under cold conditions. The positive effect of temperature increase on microbial activity was shown by the drop in ORP at all three HRT, from week ≈ 116 onwards, when temperature rose up to 15 °C.

### 5.4.2 Factors affecting Nitrate Removal Efficiency (NRE) and Nitrate Removal Rates ($R_{NO3}$)

Since denitrification is a biological process, its potential is closely related to a variety of factors (e.g., pH, ORP, organic carbon availability, temperature) affecting microbial activity (Fiedler et al., 2007; Reddy and DeLaune, 2008). In the bioreactors, the relatively stable pH ≈ 7.60 and the range of ORP values (almost always < +350 mV) indicated suitable conditions for denitrification throughout the experiment (Reddy and

DeLaune, 2008). However, many other factors could modulate microbial activity and hence influence $R_{NO3}$ and NRE. Among these factors are included temperature, HRT, concentration of $NO_3^-$-N in the initial brine, woodchip age, and salinity (Robertson, 2010; Li et al., 2017; Ghane et al., 2018).

Temperature had a clear effect on nitrate removal, particularly at week ≈ 49 and onwards when the efficiency began to oscillate following temperature oscillations (Figures 5.5A). Changes in $R_{NO3}$ and NRE values generally tracked with changes in temperature. A higher dependence of $R_{NO3}$ on temperature as woodchips age has been explained by the lower quality of organic carbon produced from woodchips (Robertson, 2010; Xu et al., 2012). In this experiment, values of the $Q_{10}$ coefficient were 1.06 ± 0.021 between weeks 1 and 53 ($R_{NO3}$ ≈ 21 g N $m^{-3}d^{-1}$ y NRE ≈ 88 %), and 1.77 ± 0.067 between weeks 54 and 121 ($R_{NO3}$ ≈ 16 g N $m^{-3}d^{-1}$ y NRE ≈ 66 %), showing a greater dependence on temperature during the second period than in the first one when woodchips were fresh, in agreement with Maxwell et al. (2020a).

The temperature dependence of nitrate removal was also observed by other authors such as Halaburka et al. (2017), that found that temperature explained 50 % of the variability in woodchip denitrification rates. Addy et al. (2016) summarized several published studies about denitrifying bioreactors in which $R_{NO3}$ increased at higher temperature ranges. They reported $R_{NO3}$ values between ≈ 2.1 and ≈ 5.7 g N $m^{-3}$ $d^{-1}$ at a temperature range between ≈ 6 and ≈ 17 °C and $R_{NO3}$ values ≈ 8.6 g N $m^{-3}$ $d^{-1}$ at temperature > ≈ 17 °C. Von Ahnen et al. (2016a) obtained $RNO_3$ values between 6.24 and 8.40 g N $m^{-3}$ $d^{-1}$ with a temperature between 7.0 to 9.6 °C, and Greenan et al. (2009) $R_{NO3}$ values between 2.9 and 4.5 g N $m^{-3}$ $d^{-1}$ with a temperature of 10 °C. In our experiment we found an average $R_{NO3}$ of 18.9 ± 0.72 g N $m^{-3}$ $d^{-1}$ (maximum 37.4 g N $m^{-3}$ $d^{-1}$) with a daily average temperature of 18.3 ± 0.54 °C, similar to values obtain by Hoover et al. (2015), who at 20 to 21.5 °C reached a $R_{NO3}$ between 10 - 21 g N $m^{-3}$ $d^{-1}$. By contrast, Warneke et al. (2011) reached an average of 7.63 ± 0.88 g N $m^{-3}$ $d^{-1}$ with temperatures between 15.5 and 23.7°C, with the highest $R_{NO3}$ of 11.2 g N $m^{-3}$ $d^{-1}$ at 23.7 °C. The cited studies show the importance of temperature for woodchips denitrifying bioreactors performance and point that these systems can be particularly suitable in warm climates such as southeastern Spain.

The HRT is another key factor for nitrate removal performance, since it must be long enough for the microorganisms to carry out the denitrification process, obtaining the necessary energy through solubilization and consumption of the organic substrate (Cooke et al., 2001; Addy et al., 2016). During periods of greater microbial activity (e.g., warm temperatures), a lower HRT would be necessary to allow enough denitrification. Robertson and Blowes (2000) (in a pilot-scale drainage with an inflow of 4.8 mg $NO_3^-$-N $L^{-1}$, 1.9 $m^3$ bioreactor and a temperature between 2 to 20 °C) and Christianson and Helmers, (2011) (in a field scale drainage with an inflow between 7.03 to 13.11 mg $NO_3^-$-N $L^{-1}$, 102 $m^3$ bioreactor and a temperature between 3 to 15 °C) concluded that an HRT < 8 h was enough to achieve NRE of ≈ 60 %. By contrast, Greenan et al. (2009) (in a laboratory scale drainage with an inflow between 50 mg $NO_3^-$-N $L^{-1}$, 0.01 $m^3$ bioreactor and a mean temperature of 10 °C) needed almost 4 days to reach the same efficiency when also treating agricultural drainage. The data obtained in our experiment show that in the first year (≈ 48 weeks), 10 h HRT was enough to remove most of the $NO_3^-$-N (NRE ≈ 75 %) in the brine during warmer periods (comparable to NRE seen at 24 h HRT), but from week ≈ 49 onwards (beginning of the second year) the NRE at 10 h HRT decreased and was lower than NRE at 24 h HRT until week 94 (end of the experiment), regardless of temperature. The high NRE values (> 80 %) during the first ≈ 48 weeks at 24 h HRT even in colder periods could have been caused by the initial DOC flush from the fresh woodchips, and would explain why denitrification was not as affected by temperature due to the high availability of organic carbon for microorganisms. This is consistent with Brettar et al. (2002), who saw high nitrate reduction coupled with high availability of organic matter and low ORP, with nitrate removal mostly independent of temperature.

Research about the role of salinity in denitrification has provided variable results. Lay et al. (2010) found that salinity decreased denitrification by affecting microorganisms in maintaining their osmotic pressure balance. However other researchers did not find apparent drawbacks for denitrifying microorganisms in saline environments (Reddy and DeLaune, 2008; Trögl et al., 2011; Álvarez-Rogel et al., 2016).

The present experiment was not designed to evaluate the effect of salinity on $R_{NO3}$ and NRE. Despite this, high nitrate removal was consistently observed throughout the 121 weeks experiment, even if rates were not constant, while salinity of the initial brine was high and fairly stable throughout (≈ 17 dS $m^{-1}$). It is reasonable to assume that microorganisms were not so hindered by the high salinity (von Ahnen et al., 2019). Furthermore, Maxwell et al. (2020b) found an increase in $R_{NO3}$ when salinity of brine increased in bioreactors with similar woodchips, although the experiment was performed over a much shorter duration (9 weeks). The current study shows that nitrate removal in woodchip bioreactors treating brine from desalination can be sustained for at least a period of 121 weeks without replacing the woodchip media.

## 5.5. Conclusions and guidelines for management

Our results showed that woodchips bioreactors are a suitable option for denitrification of nitrate-enriched brine despite its high salinity. In the Campo de Cartagena, the warm climate would favor high N removal efficiency in these systems operating at 24 h HRT, at least during the first 94 weeks (≈ first 2.5 years of bioreactors operation). Furthermore, the high DOC availability in the citrus woodchips during the first months (first 48 weeks) resulted in high NRE even at 10 h HRT. While this higher NRE during the initial weeks does not represent the long-term N removal performance, using fresh woodchips could be used as a means for achieving high NRE even at low HRT. Use of fresh woodchips would have its own drawbacks since an excess of DOC in the effluent may present challenges during discharge, particularly if discharge limits exist for organic carbon. Prior washing of fresh woodchips could be used to reduce the risk of high DOC in early denitrified brine, separating this early DOC leaching period from the brine denitrification. This washing could be done with freshwater produced by desalination while still using the leachate-rich discharge for crop irrigation.

The extremely low ORP values reached during the first months, even at 10 h HRT, indicate that sulfide formation must also be considered during brine denitrification, due to the high sulphate content of this waste. HRT must be managed to avoid significant production of reduced sulfur, mainly during the first months in which DOC leaching from woodchips is extremely high and strong anoxic conditions

are expected in the bioreactors. ORP can be an indicator of the latter and its monitoring can help to the management of this issue.

Although citrus woodchips were able to provide enough DOC for denitrification during the 2.5 years that the experiment lasted, the effect of temperature on NRE became more apparent from week ≈ 49 onwards (second year). This was likely caused by gradual wood degradation upon successive washing and indicates that, even under warm climate conditions, maintaining high NRE of the bioreactor requires active management. One option to improve performance is based on the fact that nitrate removal efficiency was highest on Mondays (first weekly batch), immediately after woodchips had been unsaturated for four days. During these four days of unsaturated conditions, it is assumed that aerobic microbial metabolism produced a flush of DOC that stimulated denitrification on the first day following re-saturation for the media (Monday in our experiment).

Developing strategies for implementing a drying-rewetting regime could improve the nitrate removal performance, particularly in colder seasons with aged wood. Use of in situ nitrate sensors could allow water quality managers to determine when sufficient NRE has been achieved and water should be discharged from the system. Less costly ORP sensors could be used instead to detect nitrate depletion and potential sulfide formation. Use of this monitoring would need to evaluate for each specific bioreactor since they are expensive and relatively difficult to manage.

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# Chapter 6

# Effect of temperature on denitrification process in woodchip bioreactors

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# Temperature sensitivity of nitrate removal in woodchip bioreactors increases with woodchip age and following drying-rewetting cycles

## 6.1. Introduction

Considering that temperature sensitivity of respiration increases with decreasing carbon quality and that Drying-Rewetting (DRW) cycles result in short-term increases in carbon quality, there should be observable changes in temperature sensitivity of $NO_3^-$ removal in woodchip bioreactors not only across long-term time scales (i.e., woodchip age over years), but also in relation to short-term dynamics following DRW cycles.

This paper uses two previously published data sets to perform a comprehensive analysis looking at the effect of the interaction between carbon quality and temperature on nitrate removal rates in woodchips. The analysis used woodchip age and time the bioreactors were empty between DRW cycles as indicators of carbon quality/availability.

## 6.2. Materials and Methods

Two published data sets were used to observe the interaction of carbon quality and temperature and its effect on $NO_3^-$ removal rates (Maxwell et al., 2018a; Díaz-García et al., 2019; Maxwell et al., 2019). The two data sets were derived from separate experiments with markedly different influent water characteristics, experimental procedures, and measurement methods. They are described briefly in the following two sections, and more detailed methods and results can be found in the cited publications. Carbon quality of woodchips or dissolved organic carbon in the bioreactor effluent was not directly measured in either study. Instead, woodchip age and elapsed time since rewetting following a DRW cycle were used as metrics for carbon quality to determine its effect on the temperature sensitivity of $NO_3^-$ removal over short and long-term time scales.

### 6.2.1 UPCT - Batch experiments treating concentrated brine

Results from three pilot-scale woodchip bioreactors treating concentrated brine were previously reported (Díaz-García et al., unpublish). Experiments were conducted at the Agri-food Experimental Station Tomás Ferro (ESEA) (N 37° 41' 17.6" and W 0° 57' 04.4") of the School of Agricultural Engineering of Universidad Politécnica de Cartagena (ETSIA - UPCT) in Cartagena, Region of Murcia, Spain. Three rectangular tanks (142 x 109 cm) were filled with chopped, citrus woodchips (depth 85 cm) obtained from agricultural sources in the region. Influent water consisted of reject brine (electrical conductivity 16 – 20 mS $cm^{-1}$, influent $NO_3^-$ concentration = 38 – 59 mg N $L^{-1}$) from a desalination plant providing irrigation water, with water sourced from an adjacent brackish aquifer contaminated with $NO_3^-$ from fertilizer and other salts (e.g., $Mg^{2+}$, $Ca^{2+}$, $Na^+$, $Cl^-$) from seawater intrusion. Bioreactors were located at the open-air facility and were therefore exposed to daily and seasonal changes in temperature. The experiment and the data obtained from it is subsequently referred to as UPCT.

Batch experiments were performed over 730 days from December 2017 to November 2019. During UPCT batch experiments, woodchip bioreactor tanks were completely-filled with brine (200 – 330 L) until water level was even with the woodchip surface. Woodchips remained fully saturated for 24 h during each batch, after which tanks were completely drained and effluent samples collected. Once the brine was removed from the bioreactors, they were immediately refilled (< 1 h after drain) and woodchips resaturated with new brine for the next 24 h batch experiment. A single batch refers to the 24 h period in which woodchips were saturated with untreated reject brine from reverse osmosis, and the denitrified brine later emptied after 24 h. Over the entire 730 d experiment, three batch experiments were performed each week beginning on Monday of each week. Following the third 24 h batch experiment of each week on Wednesday, no water was added to the bioreactors and woodchips remained unsaturated for a period of 96 h until the first batch on Monday of the following week, constituting the DRW cycle for this experiment. Data collected from first, second, and third batch runs of the week are referred to as Batch 1, Batch 2, and Batch 3, respectively.

Influent and effluent samples were collected, respectively, before and after each batch, filtered through 0.45 µm size filter (Sartorius GmbH) prior to analysis. The samples were analyzed for $NO_3^-$ concentration using double channel chromatographic system 850 Professional Ion Chromatography Metrohm at the SAIT-UPCT analytical lab in Cartagena. Concentrations of N species are reported in terms of mass nitrogen (i.e., mg N $L^{-1}$). Water temperature inside the bioreactors was measured using a Hanna handheld data logger (HI98194) with a pH/EC/temperature multiparameter probe (HI7698194) by dipping the probe into a PVC porewater well (6.3 cm) until a stable reading was reached. Batch experiments began in the early morning (t = 0 h) and finished the following morning (t = 24 h), with variable temperatures observed over each 24 h batch. Temperature values were an average of measurements taken at 30 min, 10 h and 24 h after filling the bioreactors, giving a daily average. Although diurnal temperature changes would affect microbial activity throughout the day, our aim was not to evaluate this effect but the effect of annual temperature variation (i.e., seasonal), on basis of the average daily temperature.

### 6.2.2 NCSU – Continuous flow in lab column study

The second data set used in this study was obtained from two separate lab experiments done at North Carolina State University (NCSU) investigating the effect of DRW cycles on $NO_3^-$ removal in woodchip bioreactors (Maxwell et al., 2018a, 2019). In both lab experiments, eight woodchip-filled columns (15 cm diameter x 95 cm height) were operated in continuous flow. Columns were first monitored in 2017 over a period of 287 d (Maxwell et al., 2018a) during which columns received continuous flow (HRT = 8 ± 1 h, mean ± standard deviation) from a stock tank of dechlorinated tap water dosed with $KNO_3$ (influent $NO_3^-$ concentration = 19.6 ± 1.3 mg N $L^{-1}$). A follow-up, 108 d experiment in 2018 (Maxwell et al., 2019) used the same columns with similar flow rates and influent $NO_3^-$ concentration as the 2017 experiment (HRT = 8 ± 1 h; influent $NO_3^-$ concentration = 17.1 ± 0.3 mg N $L^{-1}$). The two experiments and the data obtained from them are jointly referred to as NCSU.

In the first NCSU experiment (2017), a total of eight woodchip-filled columns were used. One treatment consisted of constant saturation (SAT) of the woodchips provided by continuous and uninterrupted upflow in four of the eight columns throughout the entire experiment. Water level in the SAT columns remained constant at the level of the column outflow, the upper surface of the woodchip media. The second treatment, performed in the other four columns, consisted of exposing the woodchips to unsaturated conditions for 8 h once a week in weekly drying-rewetting cycles (DRW) as follows; flow to DRW columns was stopped once a week by disconnecting the inflow lines, after which the DRW columns were drained rapidly (~15 min time to drain) and left unsaturated for 8 h, exposing the woodchips to unsaturated conditions. After this 8 h period where woodchips were unsaturated, flow to DRW columns was reestablished by reconnecting the inflow line. The second NCSU experiment in 2018, beginning 163 days after the end of the 2017 experiment, used four of the same columns from the prior experiment, applying the SAT and 8 h DRW treatments to two columns each. Columns reused in the 2018 NCSU experiment received the same treatment they were given in the 2017 experiment (i.e., two of the SAT columns from 2017 were also given SAT conditions in 2018). A total of 39 and 11 weekly 8 h DRW cycles were applied to the DRW treatment in 2017 and 2018, respectively. Woodchips were 558 d in age by the end of the 2018 NCSU experiment.

In both NCSU experiments, stock tank and column outflow water chemistry were measured using a small volume multiplexed pumping system (MPS)(Maxwell et al., 2018b) coupled to a high frequency spectrophotometer. The MPS sequentially pumped 25 mL samples from each column for absorbance measurement by a field spectrophotometer (Spectro::lyser; manufactured by s::can, Type SP-1-035-p0-s-NO-075) fitted with a 4 mm pathlength, 1.1 mL flow through quartz cuvette (46-Q-4, Starna Cells, Inc.). Concentrations of $NO_3^-$ in the stock tank and outflow of each column were measured on 2 h intervals. Nitrate concentrations were calculated from the absorbance measured by the spectrophotometer following methods previously described (Etheridge et al., 2014; Birgand et al., 2016). For improved accuracy of the spectrophotometer, an experiment-specific calibration was used rather than the manufacturer's calibration.

Sample volumes analyzed by the spectrophotometer were submitted for lab analysis (EPA Method 353.2, BAE Environmental Analysis Lab, North Carolina State University) to calibrate the probe for $NO_3^-$ and DOC. In the 2017 NCSU experiment, column outflow was monitored only during Days 0 – 98, 147 – 171, and 252 – 287, although columns received continuous upflow over the entire 287 days. In the 2018 NCSU experiment, column outflow was monitored over the full duration of the 108 d experiment.

Temperature of column outflow was measured hourly using Presens® temperature sensors (DP-PSt3, Presens Precision Sensing GmbH). Temperature sensors were inserted through the top of the column and placed such that the sensor tips were at least 2 cm below the surface of woodchip media, per manufacturer's specifications. Water temperature measurements were made on an hourly interval.

### 6.2.3 Nitrate removal rates

Hydraulic loading of woodchip bioreactors differed between the UPCT and NCSU experiment. Data obtained from the UPCT experiment reflect performance of bioreactors run in batch, while NCSU woodchip columns were provided continuous, uninterrupted flow outside of DRW cycles. Methods of calculating volumetric $NO_3^-$ removal rates ($R_{NO3}$), a commonly reported metric for woodchip bioreactors, were different between experiments. Volumetric rates were calculated according to Equations 1 and 2 for the UPCT and NCSU experiments, respectively:

$$\frac{([NO_3]_{in} - [NO_3]_{out}) * V_{water}}{t * V_{saturated\ woodchips}} \quad (1)$$

$$\frac{([NO_3]_{in} - [NO_3]_{out}) * Q_{10}}{V_{saturated\ woodchips}} \quad (2)$$

Where, in Equation 1, $[NO_3^-]_{in}$ and $[NO_3^-]_{out}$ are the $NO_3^-$ concentrations in the initial brine and in the effluent after 24 h, $V_{water}$ is the volume of water added to the woodchips during each batch, t is the duration of time which water was in contact with the woodchips (i.e., 24 h), and $V_{saturated\ woodchips}$ is the volume of saturated woodchips in the rectangular tanks (1.32 $m^3$). In Equation 2, $[NO_3^-]_{in}$ and $[NO_3^-]_{out}$ were the $NO_3^-$ concentrations measured at the column inlet and outlet every 2 hours, Q was the flow rate at the time of the $NO_3^-$ measurements, and $V_{saturated\ woodchips}$ is the volume of

saturated woodchips in the upflow columns (0.009 $m^3$). Removal rates were reported in units of g N $m^{-3}$ $d^{-1}$. In this study's analysis, $R_{NO3}$ was used as a metric to reflect the biogeochemical rates of $NO_3^-$ removal. For woodchip bioreactors, it has been generally assumed that denitrification is responsible for the majority of reduction in $NO_3^-$ concentration, rather than other processes such as dissimilatory $NO_3^-$ reduction to ammonium or annamox which also occur under anoxic conditions (Koop-Jakobsen and Giblin, 2010; Rambags et al., 2019). This was likely the case in both UPCT and NCSU experiments, since $NH_4^+$ concentrations in both the influent and effluent were generally less than < 2 mg N $L^{-1}$. In subsequent discussion and analysis, it is assumed that changes in $R_{NO3}$ reflected changes in denitrification rates, although the methods used in both experiments did not directly measure denitrification.

### 6.2.4 Temperature sensitivity

Temperature sensitivity of $R_{NO3}$ in both studies was quantified by calculation of the $Q_{10}$ value, or the factor by which a rate increases for every 10° C increase, a common metric used for quantifying temperature sensitivity of a biogeochemical process (Curiel Yuste et al., 2004; Zhou et al., 2009; Nordström and Herbert, 2019). Measurements of $R_{NO3}$ during each experiment were matched with corresponding temperature measurements. Data were then fitted to Equations 3 and 4 to calculate $Q_{10}$,

$$R_T = R_0 * e^{kT} \quad (3)$$

$$Q_{10} = e^{10*k} \quad (4)$$

Where $R_T$ is the observed $R_{NO3}$ (g N $m^{-3}$ $d^{-1}$) at a given temperature from measured influent and effluent $NO_3^-$ concentration, $R_0$ is a constant for the intercept, k is a constant describing the slope of the temperature relationship, and T is the measured temperature value. Collected data was fitted to the relationship in Equation 3 using the nls() function in R Studio (RStudio Team, 2020), a function finding the least-squares parameter estimates of a nonlinear function, solving for $R_0$ and k. Data from UPCT and NCSU were analyzed separately.

In the UPCT experiment, the short-term effects of carbon quality on $R_{NO3}$ temperature sensitivity were analyzed by separating data from the first, second, and third batch of each week following the 96 h unsaturated period (e.g., $Q_{10}$ for first day after DRW cycle considered only Batch 1 data). In the NCSU experiment, this short-term effect of carbon quality was analyzed by separating data according to number of days since the weekly, 8 h DRW cycle (e.g., $Q_{10}$ for first day after DRW cycle considered only data from first 24 h of continuous flow following the resaturation of the woodchips). The 2017 and 2018 data for the NCSU experiment were combined to form a single data set. The first 30 days of measurements in UPCT and NCSU experiments were removed from both data sets prior to temperature sensitivity analysis due to high amounts of organic carbon leaching in this initial period (see Section 6.3.1.1 and 6.3.2.1).

Values of $Q_{10}$ for DOC release were also calculated, substituting effluent DOC concentration into Equations 3 and 4. Standard error of the calculated $Q_{10}$ was included in the analysis, calculated as the change in $Q_{10}$ given by the standard error of the estimate for k in Equation 3. Residual standard error of the model when fitting the data to Equation 3 was used as a measure of goodness of fit.

### 6.2.5 Dynamic Q10 calculation

Uninterrupted data collection over 730 d during the UPCT experiment provided the opportunity to observe long-term changes in $Q_{10}$ over short time intervals. $Q_{10}$ was calculated dynamically over the 730 d period by subsetting the data according to time, bounded by $t_0$ and $t_1$, incrementally advancing the data window by one day at a time. Here, $t_0$ is the first day of the data window, and $t_1$ is the final day. Each $Q_{10}$ calculation consisted of 365 d of data, such that $t_1$ minus $t_0$ always equaled 365 d (i.e., separate $Q_{10}$ calculations for data collected during Day 30 – 395, 31 – 396, 32 – 397, etc.) The data window was incrementally advanced by one day at a time until $t_1$ = 730 d. Dynamic $Q_{10}$ was calculated when considering all data combined, and analyzing data from Batch 1, Batch 2, and Batch 3 separately.

### 6.2.6 Temperature dependence of Q10

Analysis of the temperature dependence of $Q_{10}$ was performed on the UPCT data set, in which average daily temperatures ranged from 8.9 – 27.8 °C. This was done by subsetting the complete data set at various temperature intervals. Each temperature interval varied by 1) minimum temperature of the interval and 2) range in temperature of the interval. For example, with a minimum temperature of 10 °C and range in temperature of 5 °C, the subsetted data for calculating $Q_{10}$ would contain only measurements from experiments in which temperatures were 10 – 15 °C. For a temperature interval with minimum temperature of 15 °C and range in temperature of 10 °C, the subsetted data would include only measurements from experiments in which temperatures were 15 – 25 °C. $Q_{10}$ was calculated by subsetting the data while varying both minimum temperature and range of the interval at increments of 1 °C. Lowest and highest values for minimum daily average temperature were 10 and 20 °C, respectively, while lowest and highest values of range in temperature were 5 and 15 °C. $Q_{10}$ was not calculated if the temperature interval contained temperatures > 25 °C (e.g., 21 – 26 °C or 15 – 27 °C). Data from Days 30 – 395 and 365 – 730 were analyzed separately. Uncertainty of the $Q_{10}$ value was calculated by using the standard error of the k coefficient when fitting the model to Equation 3.

## 6.3. Results

### 6.3.1 UPCT batch experiments

#### 6.3.1.1. Organic carbon losses from woodchips

Initial losses of dissolved organic carbon (DOC) were high in both experiments, decreasing rapidly in the first 30 days with slower long-term decreases. In the first three UPCT batch runs, mean DOC concentration in the bioreactors after 8 h was 1567 ± 195, 533 ± 44, 314 ± 45 mg C $L^{-1}$, respectively (Figure 6.1). Concentrations of DOC continued to decrease until the 12$^{th}$ batch run, after which point DOC concentrations were relatively stable. High initial flushing of DOC was the reason for excluding data from this period during $Q_{10}$ analysis. Mean DOC concentration after 24 h during the first year was 22.3 ± 10.8 mg C $L^{-1}$, with lower mean DOC in the second year of 12.1 ± 4.4 mg C $L^{-1}$. Increased DOC in the effluent was observed at warmer temperatures.

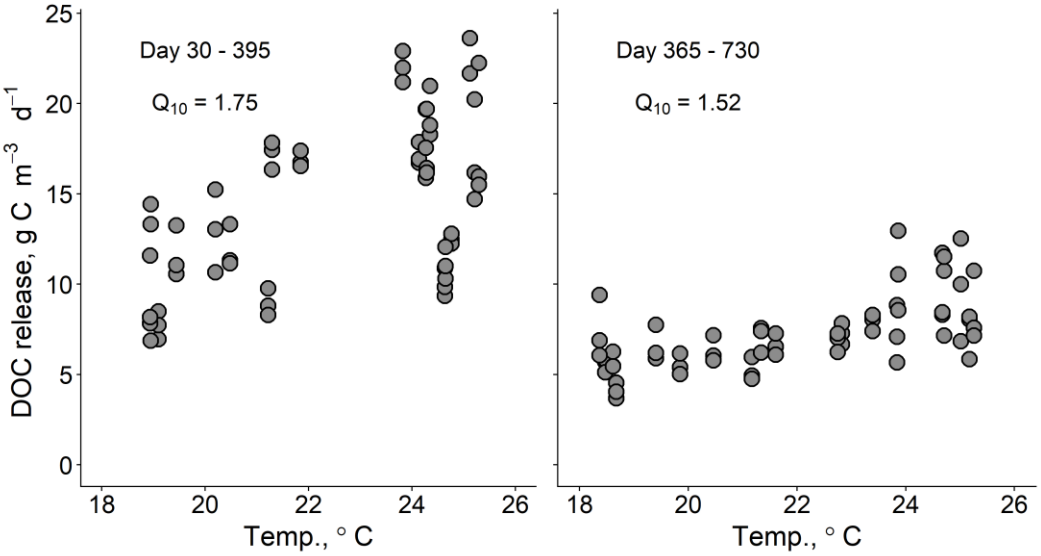


Figure 6.1. Release of dissolved organic carbon (DOC) versus temperature in UPCT bioreactors for Days 30 – 395 (first year) and 365 – 730 (second year). $Q_{10}$ of DOC release decreased with time, and values of $Q_{10}$ were positive in both periods, showing that more DOC was released at higher temperatures.

### 6.3.1.2. Temperature and $R_{NO3}$ relationships

Over the 730 d UPCT experiment, daily average temperatures ranged from 8.9 – 27.8 °C, with temperatures highest during summer months. Temperature had a clear effect on $R_{NO3}$, with large variability in $R_{NO3}$ that tracked with seasonal changes in temperature (Figure 6.2). $R_{NO3}$ was highest (up to 36.4 g N $m^{-3}$ $d^{-1}$) during the warmer summer months (24.6 ± 0.9 °C,) and lowest (as low as 7.0 g N $m^{-3}$ $d^{-}$1) during the colder winter months (12.7 ± 1.7 °C). When considering all data collected from Day 30 – 730, the k temperature constant (Equation 3) was positive and significant ($p < 0.001$), with a calculated $Q_{10}$ value of 1.71 ± 0.03 (mean ± standard deviation) and residual standard error of 4.7 g N $m^{-3}$ $d^{-1}$.

Values of $Q_{10}$ increased over the 730 d experiment (Figure 6.2). To observe long-term changes in $Q_{10}$, data were separated into three periods (representing the first year, middle of the experiment, and second year), each period 365 days in duration such that seasonal temperature variability was captured. Considering data collected from Day 30 – 395 (first year), $Q_{10}$ was 1.25 ± 0.02 with a residual standard error of 3.7 g N $m^{-3}$ $d^{-1}$. Looking at data over a one-year period during the middle of the experiment, from Day 110 – 475 (first to second year), $Q_{10}$ increased to 1.51 ± 0.03

with a higher residual standard error of 4.3 g N $m^{-3}$ $d^{-1}$. In the final year of the experiment, Day 365 – 730 (second year), $Q_{10}$ increased even further to 1.71 ± 0.03 with the lowest residual standard error of 3.0 g N $m^{-3}$ $d^{-1}$. Changes in $R_{NO3}$ over time were most noticeable at lower temperatures. During these three periods, shown in Figure 6.2, mean $R_{NO3}$ at temperatures 10 – 15 °C were 21.3 ± 5.1, 16.1 ± 5.0, and 13.7 ± 3.2 g N $m^{-3}$ $d^{-1}$, respectively. There was less variation in mean $R_{NO3}$ at higher temperatures (22 – 27 °C), with values of 27.2 ± 2.7, 27.2 ± 2.7, and 25.4 ± 2.4 g N $m^{-3}$ $d^{-1}$, respectively. Mean $R_{NO3}$ at 10 – 15 °C during Days 365 – 730 (second year) decreased by 36 %, relative to Days 30 – 395 (first year), while mean $R_{NO3}$ at 22 – 27 °C decreased by only 7 %.

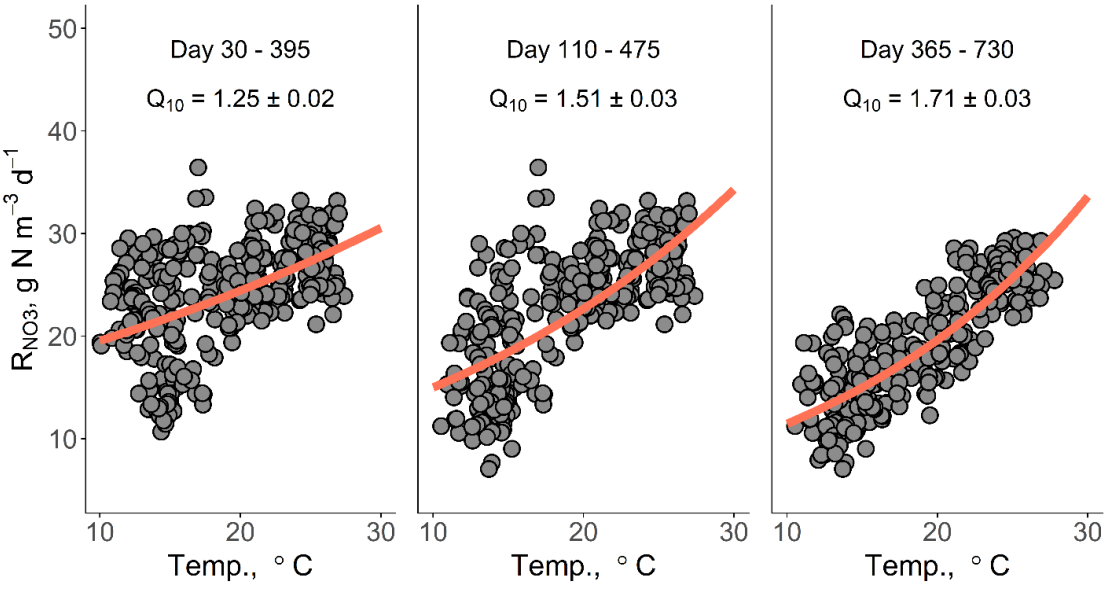


Figure 6.2 Relationship of volumetric $NO_3^-$ removal rates, $R_{NO3}$, with temperature during Day 30 – 395 (first year), Day 110 – 475 (first to second year), and Day 365 – 730 (second year) along with calculated $Q_{10}$ values (estimate ± standard error) in UPCT bioreactors. Calculated $Q_{10}$ increased over the course of the experiment, largely driven by lower $R_{NO3}$ at low temperatures as time increased.

### 6.3.1.3. Effects of drying-rewetting cycles

In the UPCT experiment, $Q_{10}$ increased with increasing number of days following the DRW cycle. It should be remembered that for the UPCT bioreactors, woodchips were exposed to 96 h of unsaturated conditions following the last batch of the week (Batch 3), with Batch 1, 2, and 3 occurring on the first, second and third day

following resaturation of the woodchips. In the first year (Day 30 – 395, Figure 6.3, black solid circles), $Q_{10}$ was lowest for Batch 1 (1.18 ± 0.02) and greatest for Batch 3 (1.34 ± 0.03). Change in $Q_{10}$ from Batch 1 to Batch 2 (0.08) was comparable to the change from Batch 2 to 3 (0.08). The same trend was seen in Day 365 – 730 (second year, Figure 6.3, hollow triangles). Batch 1 saw the lowest $Q_{10}$ (1.35 ± 0.03) with a greater difference between Batch 1 and Batch 2 (0.55). The highest $Q_{10}$ was in Batch 3 (2.01 ± 0.06). For all batches, $Q_{10}$ was greater in the second year, although the largest $Q_{10}$ increases from the first to second year were for Batch 2 (0.64) and Batch 3 (0.67).

Residual model errors for each batch were higher in the first year, at 3.2, 3.1 and 3.6 g N $m^{-3}$ $d^{-1}$ for Batch 1, 2, and 3, respectively. Residual errors in the second year decreased to 2.6, 2.4, and 2.1 g N $m^{-3}$ $d^{-1}$.

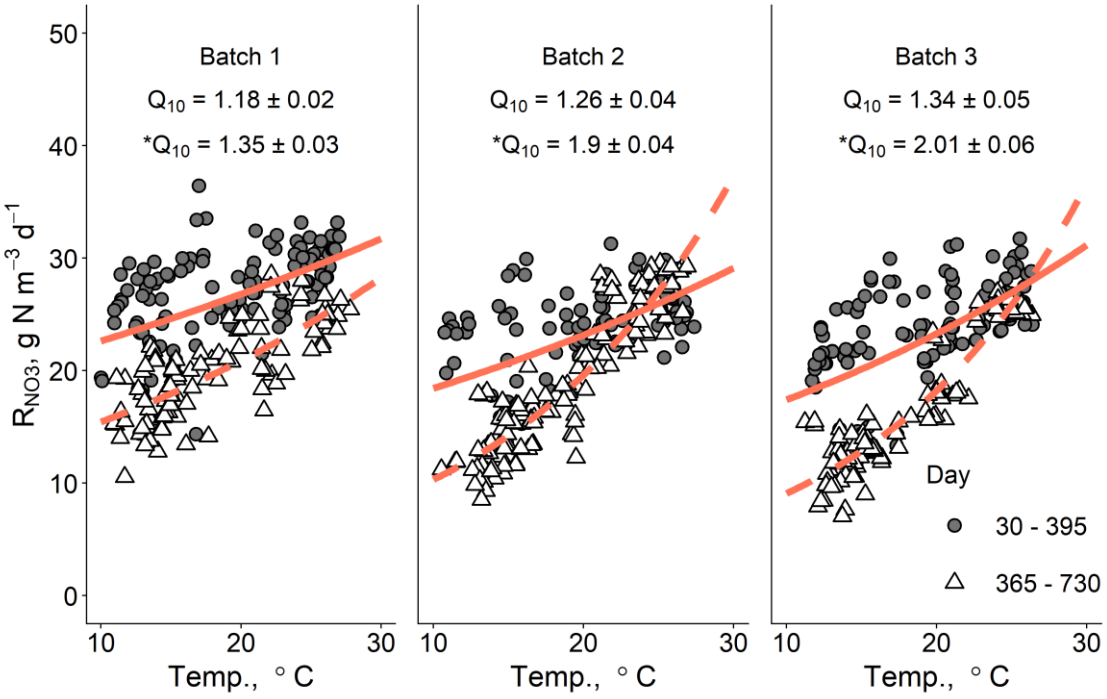


Figure 6.3. Relationship of volumetric $NO_3^-$ removal rates, $R_{NO3}$, with temperature calculated for each batch run of the week in UPCT bioreactors during Days 30 – 395 (first year, black circles, solid line) and Days 365 – 730 (second year, hollow triangles, dashed line). $Q_{10}$ values for Days 365 – 730 are denoted by the asterisk (*). In both periods, $Q_{10}$ increased with time since the DRW cycle, with higher $Q_{10}$ during the second year for all batches.

### 6.3.1.4. Dynamic $Q_{10}$ calculations

Calculated $Q_{10}$ based on data from all batches increased quickly at the beginning of the experiment from 1.25 – 1.73 over Days 50 – 155 (Figure 6.4). The $Q_{10}$ was relatively stable over Days 155 – 210, after which a slight decrease occurred. A similar initial increase over Days 50 – 150 was seen for $Q_{10}$ calculated for Batch 1, Batch 2, and Batch 3. From Days 150 – 210, $Q_{10}$ in both Batch 1 and Batch 2 were relatively stable at 1.54 and 1.72, respectively, although $Q_{10}$ for Batch 3 continued to increase slowly over Days 150 – 200. After Day 210, $Q_{10}$ for Batch 1 decreased until Day ~320, reaching a minimum of 1.32, before increasing again. $Q_{10}$ for Batch 2 began increasing on Day ~230, with the highest value of 1.90 on Day 365.

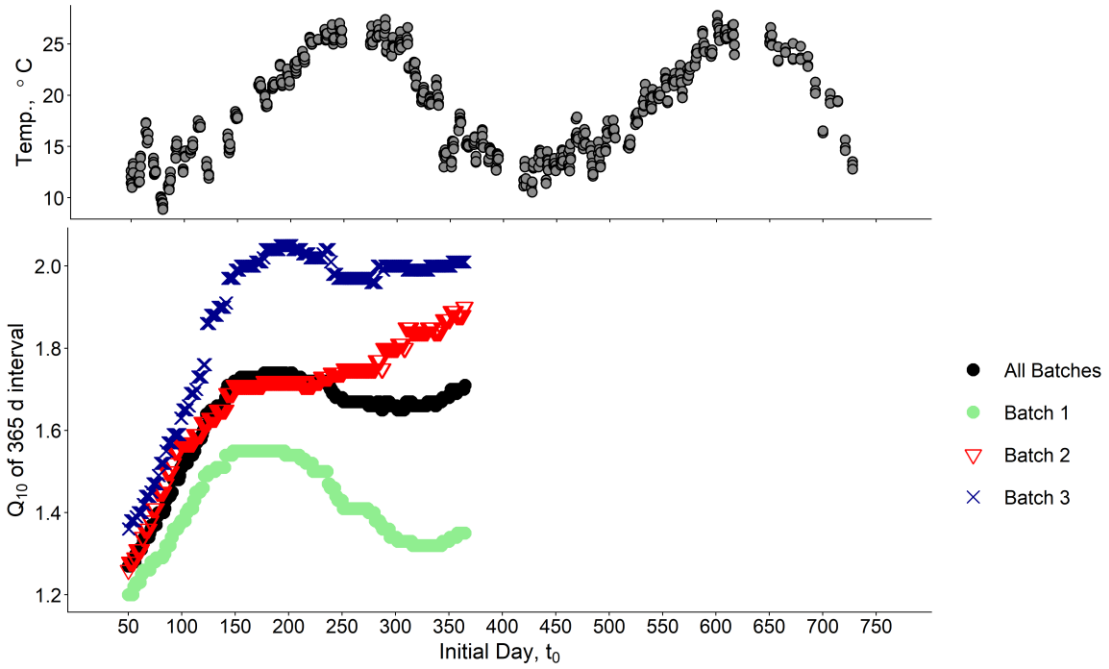


Figure 6.4. $Q_{10}$ values calculated for all batches and each batch separately for the 730 d UPCT field experiment. $Q_{10}$ was calculated dynamically over time by advancing the initial day, $t_0$, of the 365 d time window by one day at a time (i.e., $Q_{10}$ value at Day 50 on x-axis calculated using data from Days 50 – 415). Shape and color denote data from all batches or Batches 1, 2, or 3. $Q_{10}$ was not calculated after $t_0$ = Day 365 since the interval was restricted to a minimum length of 365 d. Temperature shown in the upper panel.

#### 6.3.1.5. Temperature dependence of $Q_{10}$

Subsetting the data according to temperature intervals showed variation in $Q_{10}$ values (Figure 6.5) as minimum temperature (x-axis) and range of the interval (y-axis) varied at 1 °C increments. During Days 30 – 395 (first year) values of $Q_{10}$ ranged from 1.05 – 1.51, excluding a single higher calculated value of 1.85 in subsetted data at 12 – 17 °C (Figure 6.5A). During Days 365 – 730 (second year) values of $Q_{10}$ ranged from 1.32 – 2.05 (Figure 6.5B). In both years, at a minimum temperature of 10 °C (left-most columns of tile plots), $Q_{10}$ increased as range of the temperature interval (y-axis) increased; $Q_{10}$ was 1.05 and 1.33 at 10 – 15 °C (most bottom left tile) in the first (Figure 6.5A) and second (Figure 6.5B) year, respectively, and 1.15 and 1.79 at 10 – 25 °C (most top left tile) in the first and second year. Uncertainty of the $Q_{10}$ value (calculated using the standard error of the k coefficient when fitting the data to Equation 3) was higher at smaller ranges in temperature (Figure 6.6).

For example, from Day 30 – 395, uncertainty of the $Q_{10}$ was 5.3 – 16.5 % when range of the temperature interval was 5 °C, but uncertainty was < 3 % when range of the temperature interval was greater than 13 °C. In both years, uncertainty of the $Q_{10}$ value was < 5 % when range of the temperature interval was ≥10 °C. Considering the overall $Q_{10}$ values shown in Figure 6.2 over the same time periods, analysis of the temperature dependence of $Q_{10}$ showed that $Q_{10}$ varied by up to 48 and 23 % in the first and second year, respectively, depending on the temperature interval used.

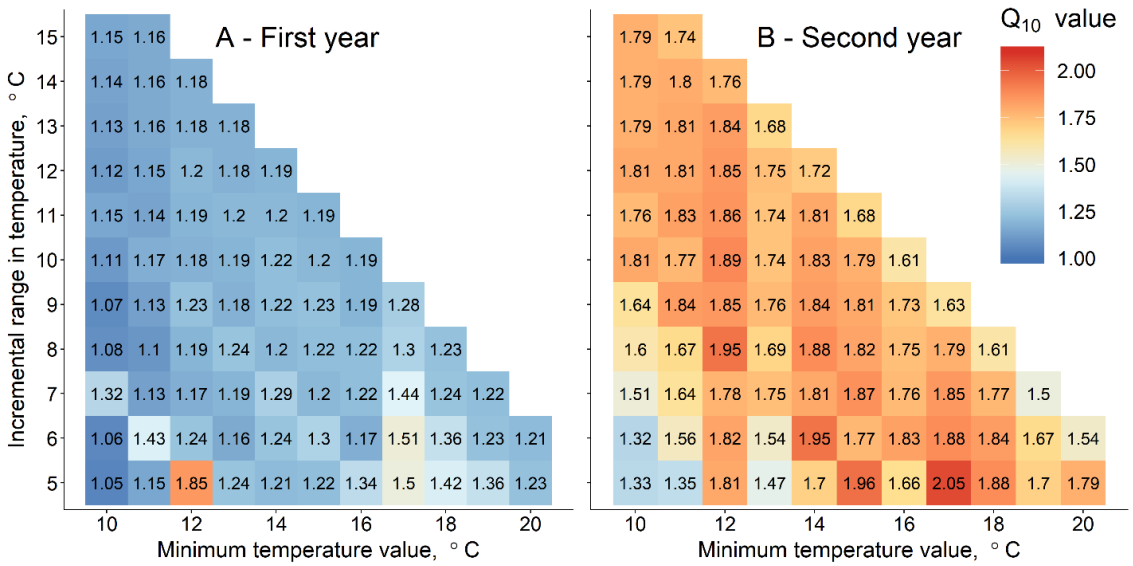


Figure 6.5. Tile plots illustrating calculated $Q_{10}$ values for the UPCT field bioreactors during Days 30 – 395 (first year, A) and 365 – 730 (second year, B). Each tile represents a separate $Q_{10}$ value when subsetting the data at various intervals according to minimum temperature (x-axis) and range in temperature of the interval (y-axis). Numbers shown within each tile are the $Q_{10}$ value at the given interval.

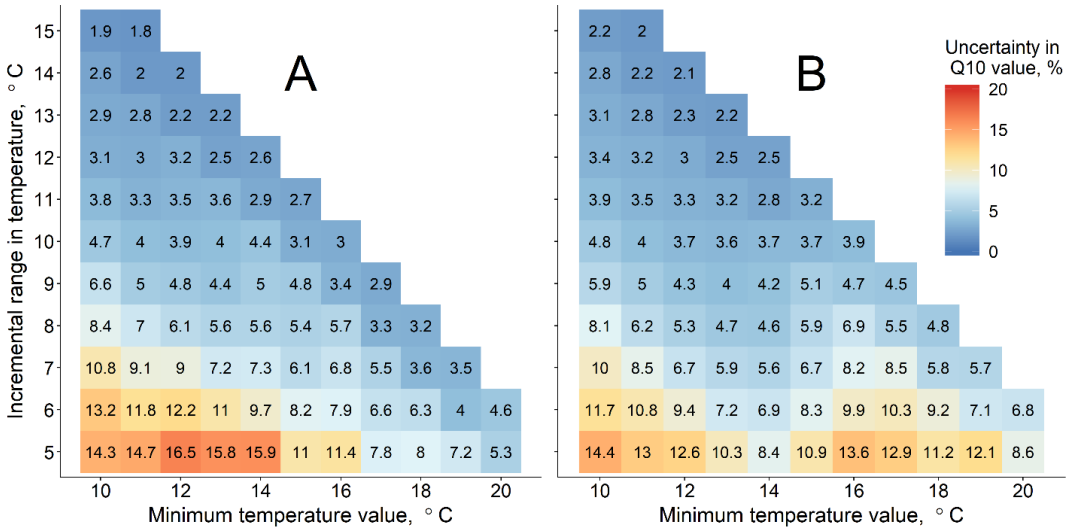


Figure 6.6. Tile plots illustrating uncertainty of the calculated $Q_{10}$ values for the UPCT field bioreactors during Days 30 – 395 (first year, A) and 365 – 730 (second year, B). Each tile represents a separate $Q_{10}$ value when subsetting the data at various intervals according to minimum temperature (x-axis) and range in temperature of the interval (y-axis). Numbers shown within each tile are the uncertainty of the $Q_{10}$ value at the given interval. Uncertainty generally decreased as range in temperature of the interval increased.

### 6.3.2. NCSU column study

#### 6.3.2.1. Organic carbon losses from woodchips

Concentrations of DOC were initially high in effluent from the NCSU columns, although values were much lower relative to UPCT batches since columns were operated in continuous flow with an ~8 h HRT. From Day 20 – 50, effluent DOC concentration was 3.4 ± 0.7 and 3.5 ± 0.7 mg C $L^{-1}$ for SAT and DRW columns, respectively (Figure 6.7). From Day 50 – 176 mean DOC was 2.8 ± 0.3 and 3.0 ± 0.4 mg C $L^{-1}$ for SAT and DRW columns, and decreased further during Day 252 – 287 to 1.5 ± 0.1 and 1.7 ± 0.2 mg C $L^{-1}$. During 2018 (Day 480 – 558), mean DOC was 1.7 ± 0.3 and 2.0 ± 0.4 mg C $L^{-1}$. Concentrations of DOC were marginally higher in DRW columns, relative to SAT, with the greatest different in DOC concentration immediately following the DRW cycle. In terms of volumetric rates of DOC release, calculated similarly to $R_{NO3}$ using Equation 2, mean rates of DOC release during Day 30 – 287 (2017) were 1.3 ± 0.7 g C $m^{-3}$ $d^{-1}$, and 1.8 ± 0.9 g C $m^{-3}$ $d^{-1}$ during Day 480 – 558 (2018).

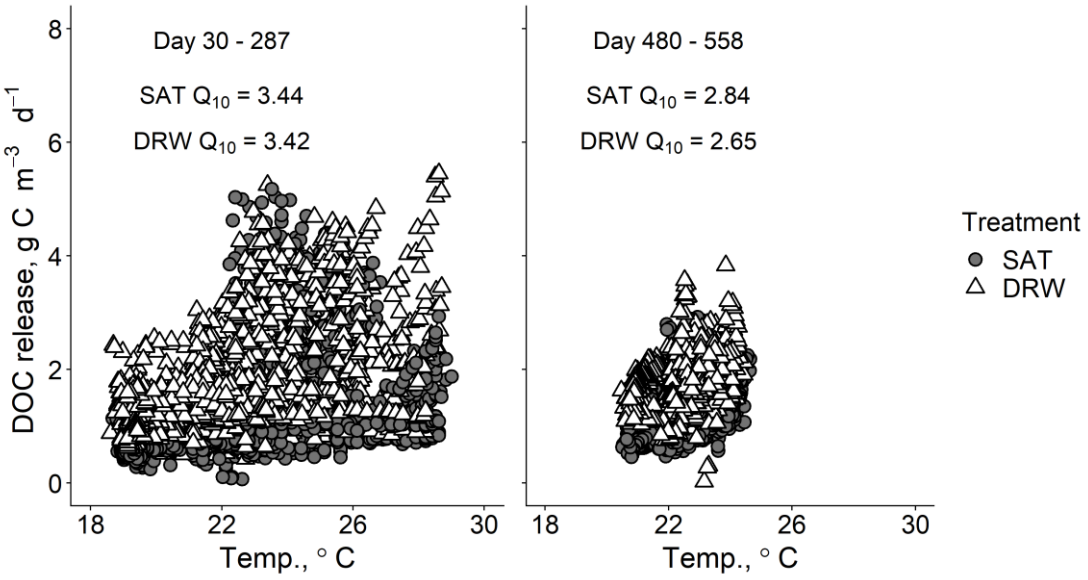


Figure 6.7. Release of dissolved organic carbon (DOC) versus temperature in NCSU bioreactors for Days 30 – 287 and 480 – 558. $Q_{10}$ of DOC release decreased with time, and values of $Q_{10}$ were positive in both periods, showing that more DOC was released at higher temperatures.

#### 6.3.2.2. Temperature and $R_{NO3}$ relationships

From Day 30 – 287 temperatures ranged from 18.6 – 29.0 °C (21.6 ± 1.9 °C), while temperatures from Day 480 – 558 ranged from 20.5 – 24.7 °C (22.7 ± 0.9 °C). Temperature had a clear effect on $R_{NO3}$ when considering data from Day 30 – 287 (2017) and 480 – 558 (2018) separately, with the k temperature constant (Equation 3) significant ($p < 0.001$) and positive during both periods. When considering all data collected from Day 30 – 558 (2017 and 2018), there was a calculated $Q_{10}$ value of 1.95 ± 0.02 and residual standard error of 3.9 g N $m^{-3}$ $d^{-1}$.

Unlike the analysis for the UPCT bioreactors, which had uninterrupted data collection over the entire 730 d period, long-term changes in $Q_{10}$ of the NCSU woodchip columns were analyzed by breaking the data into two periods only, the 2017 and 2018 portions of the NCSU experiment (each containing only 287 and 108 d of data collection, respectively). Values of $Q_{10}$ decreased over the 558 d duration of the NCSU experiment (Figure 6.8). Lower $Q_{10}$ was seen from Day 30 – 287, relative to Day 480 – 558, and $Q_{10}$ values were not significantly different between the SAT and DRW treatments. Values for $Q_{10}$ were higher during Day 480 – 558, with a larger difference in $Q_{10}$ between the two treatments.

Increase in $Q_{10}$ from Day 30 – 287 to Day 480 – 558 was higher for the DRW treatment (0.71) relative to the increase for the SAT treatment (0.25). Residual standard error of the $Q_{10}$ model from Day 30 – 287 was 3.3 and 3.8 g N $m^{-3}$ $d^{-1}$ for SAT and DRW columns, respectively, and 3.3 and 2.6 g N $m^{-3}$ $d^{-1}$ from Day 480 – 558.

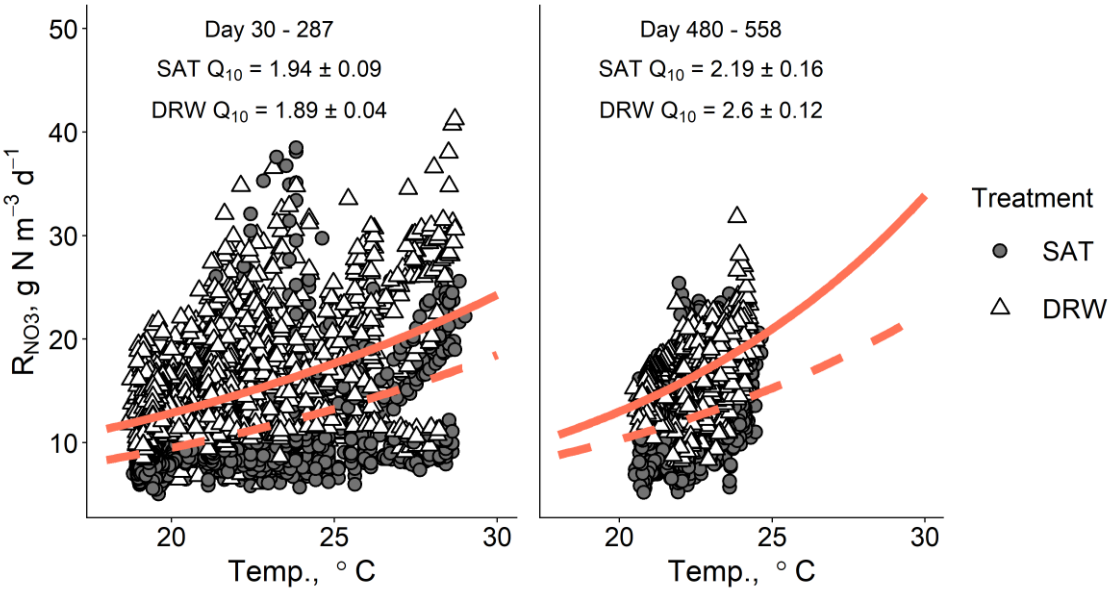


Figure 6.8. Relationship of volumetric $NO_3^-$ removal rates, $R_{NO3}$, with temperature during Day 30 – 287 (2017) and Day 480 – 558 (2018) for the NCSU column experiment. $Q_{10}$ values (estimate ± standard error) were calculated separately for SAT (dashed line) and DRW (solid line) treatments.

### 6.3.2.3. Effects of drying-rewetting cycles

Short-term increases in $Q_{10}$ were seen in the NCSU experiment (Figure 6.9) when selecting $R_{NO3}$ and calculating $Q_{10}$ separately for each day following the resaturation of the woodchips. Data were not divided by year in this analysis, and data from SAT columns were not used since the columns did not undergo a DRW cycle. In general, $Q_{10}$ increased following the weekly 8 h DRW cycle. A large increase in $Q_{10}$ was seen between Day 1 and Day 2 after rewetting (0.56) and between Day 3 and Day 4 (0.86). Daily increases in $Q_{10}$ were seen in every day until Day 5 following the DRW cycle, with a small decrease in $Q_{10}$ on Day 6. A wider range in temperature for Days 4 – 6 (18.6 – 28.7 °C) after rewetting (Figure 6.9), relative to Days 1 – 3 (18.9 – 26.8 °C), may have had an effect on the higher observed $Q_{10}$ values for Days 4 – 6.

However, $R_{NO3}$ tended to decrease at lower temperatures with increasing time since resaturation; at temperatures <20 °C, mean $R_{NO3}$ on Days 1 – 6 after rewetting were 14.2, 13.1, 11.5, 10.4, 10.5, and 11.2 g N $m^{-3}$ $d^{-1}$.

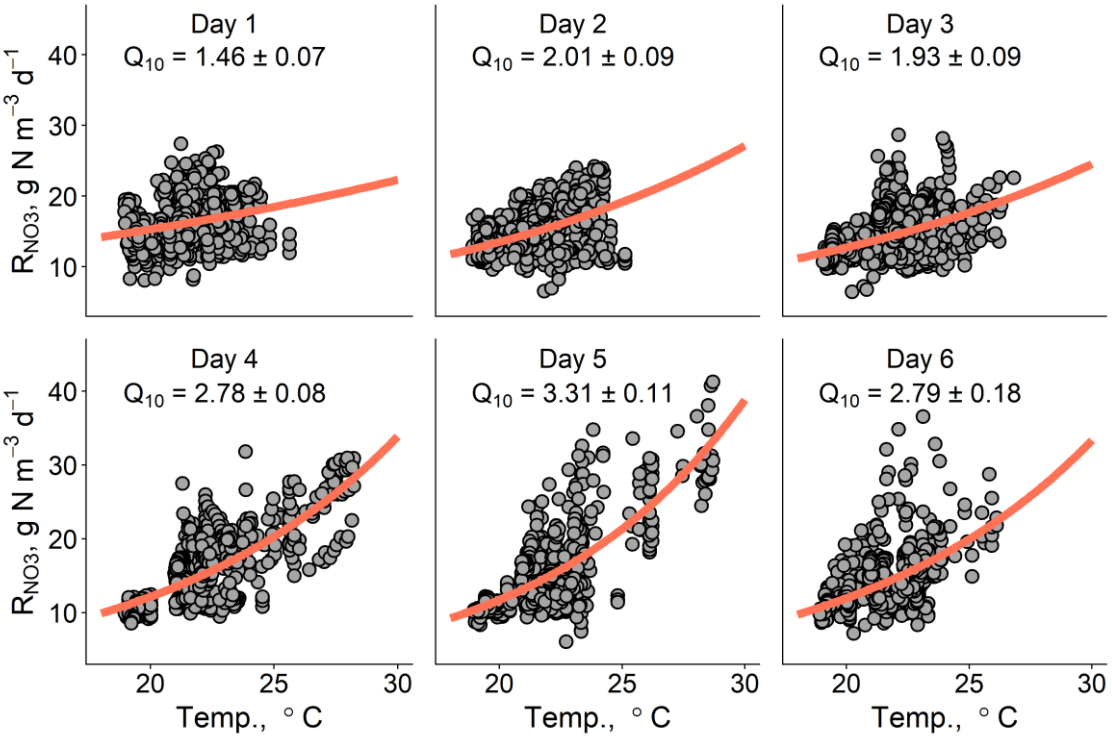


Figure 6.9. Relationship of volumetric $NO_3^-$ removal rates, $R_{NO3}$, with temperature in the NCSU experiment when separating data according to number of days since the 8 h DRW cycle (i.e., the top left panel includes only measured $R_{NO3}$ values within the first 24 h after resaturation of woodchips). Data for each day after rewetting were pooled irrespective of year (2017 and 2018 data combined).

## 6.4. Discussion

### 6.4.1 Long-term changes in $Q_{10}$

Data from both experiments support the initial hypothesis that temperature sensitivity of $NO_3^-$ removal in woodchip bioreactors increases over time. The most likely explanation for these observed long-term increases in $Q_{10}$ is changes in carbon quality of the woodchips over time. Ghane et al. (2018) showed the relative proportion of lignin in woodchips in a field bioreactor increased over time, with decreasing content of cellulose and hemicellulose. Breakdown of recalcitrant, lignin-heavy organic material through anaerobic respiration has been shown to be negligible.

This is possibly due to the inability of the anaerobic pathway to breakdown the complex linkages that occur in lignin (Koshijima and Watanabe, 2004; Talbot et al., 2012). Limited degradation of the woodchips by denitrifiers may be as much due to the carbon structure as its composition, with much of the cellulose in woody material

protected by a lignin "sheath" that is resistant to enzymatic attack (Sadaf et al., 2018). Assuming more bioavailable cellulose and hemicellulose was lost from the woodchips over time in the UPCT and NCSU experiments, denitrifiers were less efficient at metabolizing the remaining carbon to achieve reduction of $NO_3^-$ to gaseous N. Changes in the $Q_{10}$ value were mostly driven by decreased $R_{NO3}$ at lower temperatures, rather than increases in $R_{NO3}$ at higher temperatures, suggesting that denitrification rates at higher temperatures were less affected by changes in carbon quality. Declines in nitrate removal rates in aged woodchips at low temperatures is an important aspect of woodchip bioreactors that should be considered for their use in cold weather climates. For example, woodchips bioreactors have been widely adopted in the Midwest United States as a water quality BMP for $NO_3^-$ load reductions in drainage water. Temperature of tile drainage water in this region, however, is low for most of the year, particularly during the months of April – May (4 – 10 °C) (David et al., 2016) when as much as 40% of annual tile flow can occur (Helmers et al., 2005). The highest losses in efficiency for woodchip bioreactors over time will occur at the lowest temperatures.

While the increasing $Q_{10}$ values can be considered an indicator of decreasing carbon quality, a separate indicator was the residual model error of the $Q_{10}$ relationship when fitting the relationship in Equation 3. In the UPCT experiment, this residual model error decreased over time from 4.3 g N $m^{-3}$ $d^{-1}$ during Day 30 – 395 to 3.0 g N $m^{-3}$ $d^{-1}$ during Day 365 – 730. A similar trend was observed in the NCSU data from Day 30 – 287 (2017) to Day 480 – 558 (2018), where model error did not change in the SAT group but decreased from 3.8 to 2.6 for the DRW columns. Change in the model error can illustrate temperature sensitivity of $NO_3^-$ removal, as more of the $R_{NO3}$ variability was able to be explained by temperature only when carbon quality was low. A simple temperature-dependent relationship was less capable of explaining $R_{NO3}$ variability when carbon availability was high.

Temperature only explained 54 – 85 and 26 – 47% of $R_{NO3}$ variability in the UPCT and NCSU experiments, respectively, indicating there were likely additional factors (e.g., carbon availability) affecting $NO_3^-$ removal rates.

### 6.4.2 Effect of drying-rewetting cycles on $Q_{10}$

Drying-rewetting cycles had both short and long-term effects on $Q_{10}$. In the NCSU experiment, change in $Q_{10}$ from the Day 30 – 297 to Day 480 – 558 in constantly saturated SAT columns was low (0.25). This contrasted with the larger change in $Q_{10}$ for DRW columns (0.71), as the weekly aerobic periods would have resulted in greater degradation of and carbon loss from the woodchips. The short-term effect was also apparent, as $Q_{10}$ generally increased with each subsequent day after woodchips were resaturated. This was most likely caused by the gradual flushing or consumption of aerobically-produced DOC following the DRW cycle, consistent with previous findings showing DOC leaching highest immediately following DRW cycles and decreasing quickly (i.e., within days) after resaturation (Groffman and Tiedje, 1988; Gordon et al., 2008; Beare et al., 2009). Byproducts of incomplete decomposition of organic matter (e.g. DOC) are typically lower molecular weight electron donors (Fox and Comerford, 1990; Van Hees et al., 2005; Lützow et al., 2006), with lower molecular weight organic compounds more bioavailable for certain microbes (Cleveland and Townsend, 2006; Cleveland et al., 2007; Eilers et al., 2010). The DRW cycles exposed the lignin-heavy woodchips to aerobic conditions while the media was unsaturated, producing more labile carbon as a result of the more rapid aerobic degradation. Once the media was resaturated and anaerobic conditions resumed, denitrifiers had access to higher quality carbon which led to higher $R_{NO3}$.

The effect of the DRW cycle was also apparent in the UPCT experiment (Figure 6.3). During Days 30 – 395, $Q_{10}$ following the 96 h unsaturated period changed with number of days following resaturation, with the greatest $Q_{10}$ in the third batch run of the week. The same was true during Days 365 – 730, with larger increases in $Q_{10}$ between consecutive batches. Degree of decomposition of the UPCT woodchips during Days 365 – 730, after the fresh woodchips had been used for one year, would be most comparable to the aged NCSU woodchips.

There was a large increase in $Q_{10}$ between Batch 1 and Batch 2 during Days 365 – 730 in the UPCT bioreactors (0.55, Figure 6.3, hollow triangles), comparable to the increase in $Q_{10}$ from Day 1 to 2 in the NCSU experiment (0.56, Figure 6.9). Similarly, the increase in $Q_{10}$ from the second to third day, in both experiments, was 0.11 – 0.12,

suggesting the largest changes in carbon quality occurred in the first 24 h following the DRW cycle as aerobically-produced carbon was leached or consumed. Residual model errors fitting the data to Equation 3 also decreased with time since the DRW cycle for both experiments. Using the $Q_{10}$ values from Day 365 – 730 of the UPCT data (Figure 6.3, hollow triangles) and the NCSU data (Figure 6.9), the relationship of $Q_{10}$ versus number of days since rewetting was well-fitted by a natural log equation of $Q_{10} = 0.62 * \ln(t) + 1.38$ ($R^2$ =0.95) for UPCT and $Q_{10} = 1.05 * \ln(t) + 1.18$ ($R^2$ =0.90) for NCSU, where *t* is number of days since rewetting.

Higher carbon quality and/or availability can explain the observed long-term increases in $Q_{10}$ as woodchips aged (Figure 6.2 and 6.8) and with elapsed time since a DRW cycle (Figure 6.3 and 6.9). Denitrifiers would have had greater access to more labile carbon when woodchips were less aged (i.e., higher cellulose content) and immediately following unsaturated periods that made lower molecular weight carbon more available via aerobic processes. Once woodchips were resaturated, and anaerobic conditions reestablished, higher denitrification rates would be observed due to the greater carbon availability. This hypothesis attributes differences in carbon availability solely to changes in quality of the woodchip-derived carbon directly accessible to denitrifiers. This differs from the conclusion previously reached by Nordström and Herbert (2019), which also saw long-term increases in $Q_{10}$ for $NO_3^-$ removal in woodchip bioreactors. The authors concluded changes had occurred in the microbial community composition and/or the degree of cross-feeding between denitrifiers and fermenting bacteria. This was based on the authors' assumption that denitrifiers in woodchip bioreactors rely on the byproducts (e.g., sugars, volatile fatty acids, $H_2$) of upstream fermenters for electron donors. Although it has been shown that cross-feeding between fermenters and denitrifiers occurs (Hanke et al., 2016), it is possible that there are other mechanisms explaining the increase in temperature sensitivity of denitrification over time.

The present study suggests a separate hypothesis, independent of fermentation activity, that accounts for these long-term changes in $Q_{10}$. A significant portion of fresh woodchips is comprised of cellulose (35 – 56%) (Rowell, 2012; Christianson et al., 2016; Feyereisen et al., 2016), relative cellulose content of woodchips decreases over time (23 – 31% after four years) (Ghane et al., 2018), and, in an oxygen-free environment, a pure culture of denitrifiers is capable of using cellulose as a carbon source (Godini et al., 2011). This rationale for the long-term change in $NO_3^-$ removal rates is consistent with the previously established carbon quality-temperature hypothesis, that respiration rates are increasingly sensitive to temperature as carbon quality of the organic matter decreases. This hypothesis also explains the observed short-term changes in $Q_{10}$ immediately following a DRW cycle, since carbon availability would be highest immediately following the unsaturated period in which aerobic processes likely occurred. It is possible that either or both processes (i.e., cross-feeding of fermenters and denitrifiers, short/long-term changes in carbon quality of the media) are occurring in woodchip bioreactors.

Although the present study observed changes in $Q_{10}$ in response to DRW cycles in a high C content substrate (i.e., woodchips), the results are applicable to understanding processes driving organic decomposition in soils. The fact that elapsed time since resaturation of woodchips had an impact on $Q_{10}$ may help explain variability in the literature regarding $Q_{10}$ for respiration of organic matter. A number of studies have indicated that factors other than carbon quality must be driving changes in $Q_{10}$ (Ise and Moorcroft, 2006; Craine and Gelderman, 2011; Reynolds et al., 2017). Peaks in denitrification rates can occur immediately following DRW cycles upon rewetting (Beare et al., 2009). Changes in moisture content via DRW cycles, exposure of carbon to aerobic breakdown, and subsequent leaching of soluble organics could explain the variability of $Q_{10}$ in the literature that cannot be explained by carbon quality alone.

### 6.4.3 Woodchip degradation and carbon availability

Several factors could cause woodchips to degrade at different rates, and simply using the age of woodchips to predict $Q_{10}$ over time may not be accurate. Moorman et al. showed that shallower woodchips more frequently exposed to aerobic conditions in a field bioreactor had 55% greater carbon loss relative to those in deeper woodchips (Moorman et al., 2010). In the UPCT experiment, woodchips were exposed to a 96 h unsaturated period once each week. It is possible that woodchips exposed to shorter unsaturated periods would have a lower increase in $Q_{10}$ from the first to second year, relative to the 0.46 increase seen in UPCT bioreactors (Figure 6.2). However, despite DRW columns in the NCSU experiment being exposed to a much shorter 8 h DRW cycle, relative to the UPCT bioreactors, they saw a larger increase in $Q_{10}$ (0.71) from the first to second year. This may have been due to the fact that NCSU columns were operated in continuous flow, rather than in batch experiments. Continuously receiving aerated water (~ 8 mg DO $L^{-1}$) may have caused NCSU woodchips to degrade faster than if they had been operated in 24 h batch experiments. Woodchips in NCSU columns were also left unsaturated between the 2017 and 2018 experiment, and likely experienced greater rates of degradation over this period. A third factor that may have increased the rate of degradation of the UPCT woodchips was the use of saline brine in which sodium concentrations ranged from 2,600 – 5,000 mg Na $L^{-1}$. Previous research has shown that high salinity (Marton et al., 2012) or sodicity (Steele and Aitkenhead-Peterson, 2013) can increase the breakdown of organic matter. Indeed, previous experiments at the UPCT facility showed DOC in the effluent of woodchips was greater as brine became more concentrated. Changes in temperature sensitivity over time would be site specific and depend on various factors, including degree of exposure to aerobic conditions and water chemistry.

In both experiments, rates of DOC release increased at higher temperatures. Values of $Q_{10}$ for DOC release during Days 30 – 395 and 365 – 730 of the UPCT experiment were 1.75 and 1.52, respectively (Figure 6.1); $Q_{10}$ of DOC release for NCSU SAT and DRW groups were 3.44 and 3.42 during Days 30 – 287 and 2.84 and 2.65 during Days 480 – 558 (Figure 6.7). While part of the temperature response of $R_{NO3}$ would have been related to the efficiency of denitrifiers to metabolize carbon, the

effect of temperature may also have been confounded with higher aerobic decomposition rates when woodchips were unsaturated resulting in greater carbon availability, linking the temperature sensitivity of aerobic and anaerobic respiration. $Q_{10}$ values for denitrification in woodchip bioreactors combine the effect of several processes which are also affected by temperature, such as those which increase carbon availability of woodchip-derived carbon (i.e., aerobic breakdown during unsaturated conditions).

Increased C availability at higher temperatures due to aerobic breakdown may explain dynamic trends in $Q_{10}$ during the UPCT experiment. The overall $Q_{10}$ and $Q_{10}$ for each batch run increased until Day ~140 – 150 at which point $Q_{10}$ values reached a plateau (Figure 6.4). Subsequently, $Q_{10}$ for Batch 1 began to decrease after Day ~210, while $Q_{10}$ increased for Batch 2 roughly 20 days later as temperatures were increasing during the summer months. This could be explained by greater carbon availability via more efficient aerobic breakdown at warmer temperatures, with denitrifiers able to consume nearly all of the aerobically-produced carbon during Batch 1 and leaving less available for the subsequent Batch 2. The fact that most of the increase in $Q_{10}$ for all batches occurred during the first ~150 days is consistent with previous findings that most of the declines in $NO_3^-$ removal in woodchip bioreactors occurs relatively rapidly (< 1 year) and is relatively stable after this initial leaching period of more readily consumed carbon (i.e., cellulose and hemicellulose). It is also possible that changes in the microbial community during Days 150 – 230 that caused $Q_{10}$ changes in Batch 1 and 2.

### 6.4.4 Temperature dependence of $Q_{10}$

Several studies have reported higher (Hoover et al., 2016) or lower (Nordström and Herbert, 2019) $Q_{10}$ values at higher temperatures, and that $Q_{10}$ can depend on magnitude of or total range in temperature observed (Tjoelker et al., 2001). During Days 365 – 730 (second year), at minimum temperature values of 10 to 11 °C (x-axis), calculated $Q_{10}$ generally increased with increasing range in temperature values (y-axis), indicating data collected at low temperatures over a small range in temperature may bias $Q_{10}$ values towards underestimation. This is possibly due to the fact that variability in observed rates (as affected by measurement uncertainty or experimental variability)

are larger relative to the total temperature-induced change in rates, when temperature range is small. This was seen in the higher uncertainty of the $Q_{10}$ values at smaller ranges of the temperature interval (Figure 6.6, y-axis), which may have explained the higher variability in $Q_{10}$ values at different values of minimum temperature when the range in temperature of the interval was only 5 – 6 °C (bottom two rows of tile plots in Figure 6.8). In both years of the UPCT experiment, uncertainty of $Q_{10}$ was < 5 % when the range in the temperature interval was ≥ 10 °C. Researchers calculating $Q_{10}$ values when range in temperature is small should consider this additional uncertainty when drawing conclusions.

Using the results of this study as an example, temperature range during Day 480 – 558 of the NCSU experiment (20.5 – 24.7 °C) was smaller than during Day 30 – 287 (18.6 – 29.0 °C). Recalculating $Q_{10}$ during Day 30 – 287, subsetting the data to the same temperature interval seen during Day 480 – 558, $Q_{10}$ values were 1.41 and 1.27 for SAT and DRW groups, respectively, indicating that the change in $Q_{10}$ from the first to second year may have been much greater than initially thought (Figure 6.8). Additionally, in NCSU bioreactors there was a lower total range in temperature seen in Days 1 – 3 after rewetting (19 – 25 °C, Figure 6.9), relative to Days 4 – 6 after rewetting (19 – 29 °C). Recalculating $Q_{10}$ values by subsetting the data to the smaller temperature range (19 – 25 °C), $Q_{10}$ for Days 1 – 6 were 1.53, 2.11, 2.07, 3.37, 3.83, and 2.79, respectively, which still showed an increase in $Q_{10}$ with number of days since resaturation of the woodchips.

## 6.5. Conclusions and management considerations

Temperature sensitivity of $NO_3^-$ removal rates in woodchips bioreactors increased as woodchip aged in both experiments, showing that woodchip age is an important parameter in understanding the effect of temperature on $NO_3^-$ removal and when calculating $Q_{10}$. Similarly, DRW cycles caused brief increases in $NO_3^-$ removal that tended to decrease temperature sensitivity immediately after rewetting, which was modulated by time elapsed since the DRW event, as shown by higher $Q_{10}$ values as time since resaturation increased. Both trends can be attributed to decreasing bioavailability of carbon for anaerobic denitrification and are consistent with the carbon quality-temperature hypothesis.

Soluble organic carbon in the effluent also increased at higher temperatures, particularly after DRW cycles, which was coincidental with increases in $NO_3^-$ removal rates. This finding suggested that microbial activity was stimulated at higher temperature during unsaturated conditions, leading to a surplus of low-molecular weight soluble organic carbon compounds via incomplete respiration, which, in turn, may have played some part in the temperature response of denitrification during the subsequent flooding phase. Although it is clear that DRW cycles produce increased nitrate removal rates, the management method is likely to lead to more rapid degradation of the media. Implementing DRW cycles may also require additional resources (e.g., equipment, labor) to regularly drain and resaturate media. Water quality managers would need to consider these factors when choosing between a continuously saturated system or one with intermittent DRW cycles.

Short and long-term changes in temperature sensitivity in woodchip bioreactors should be considered both in the context of agricultural water management and its behavior under changing climactic conditions. Water quality planners should consider declines in $NO_3^-$ removal efficiency over time will be greatest at lower temperatures. Similarly, depending on regional impacts of climate change, more prolonged dry periods would lead to greater degradation under unsaturated conditions of woodchips since field woodchip bioreactors are often located above the water table and drainage lines.

## 6.6. Acknowledgements

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# Chapter 7

# Woodchip bioreactors to denitrify saline effluents from intensive agriculture

The content of this chapter is in preparation to be published:

Díaz-García, C., J.J. Martínez-Sánchez, and J. Álvarez-Rogel. Woodchip bioreactors to denitrify saline effluents from intensive agriculture in a Mediterranean semiarid watershed.

# Woodchip bioreactors to denitrify saline effluents from intensive agriculture in a Mediterranean semiarid watershed

## 7.1. Introduction

Despite being a dryland area, the Campo de Cartagena is one of the main agricultural fertigation areas of Europe with 40 000 hectares of irrigated land. During the last 40 years, farmers have used fertilizer which has been leached and filtered to the ditches and ramblas (ephemeral surface watercourses), discharging to the Mar Menor lagoon and infiltrating to the aquifer. To minimize the impacts of these leachates, agricultural best management practices should be implemented for a more sustainable development with long-term goals, in agreement with available resources and minimizing the environmental impacts. Additionally, polluted water discharged to the Mar Menor via various hydrologic pathways (hydrologic network, subsurface flow, drainage ditches, etc.) must be captured and treated. The latter could be achieved with the implementation of nature-based solutions (NBS) in the watershed, which are defined by natural ecosystem functions that include processes of natural attenuation normally involving microbial removal of pollutants from water systems (Cohen-Shacham et al., 2016), and are considered alternatives to man-made infrastructures that require large investment in materials and energy (Nesshöver et al., 2017). Wetlands and woodchip bioreactors are considered examples of NBS (Thorslund et al., 2017).

After demonstrating the efficiency of woodchips bioreactors for brine denitrification under batch regime, a new experiment was carried out to assess the efficiency of citrus woodchips bioreactors working under continuous flow regime for reducing the high $NO_3^-$ loads from agricultural leachates flowing in surface water courses of the Campo de Cartagena. This study aimed to get a preliminary assessment of how different hydraulic residence times affect woodchips degradation and assess the existence of potentially harmful substances in the bioreactor effluents.

## 7.2. Materials and methods

### 7.2.1 Experimental set up

A 566 days (≈80 weeks, 29 May 2019 to 17 November 2020) pilot scale experiment was conducted in a Pilot Plant with denitrifying bioreactors located at the Los Alcázares Urban Wastewater Treatment Plant (UWWTP) (N 37°44'31.14" and W 0°53'3.52"), Region of Murcia, Spain.

Water treated by the bioreactors was pumped to a unique distribution drum from the nearby D7 drainage ditch before being fed to the bioreactors. The D7 ditch is one of the main channels collecting agricultural drainage water and leachates of the Campo de Cartagena, although it can also receive other effluents (upwelling water from the Quaternary aquifer and point discharges from other UWWTP located upstream). The bioreactors consisted of three rectangular ditches (6 x 1.2 x 0.98 m) arranged in parallel, each bioreactor filled with 7 $m^3$ of citrus woodchips. To calculate bioreactor dimensions, we started from the inlet flow of the impulsion pump (9 $m^3$ $d^{-1}$) and the citrus woodchips porosity that had been previously defined (Díaz-García et al., 2020). The bioreactors dimensions were determined by the available area in the construction area following the construction recommendations of other authors (Christianson et al., 2010; Christianson and Helmers, 2012). Once the flow rate (Q), the volume (V) of the bioreactor and the woodchips porosity (ρ) were obtained, the height of the outlet pipes was determined to target a specific theoretical hydraulic residence time (HRT), where HRT was calculated:

$$HRT = \frac{V * \rho}{Q}$$

Citrus woodchips were chosen as carbon media because they are a waste largely accessible in Mediterranean areas and because previous studies showed its efficiency for denitrification (Díaz-García et al., 2020).

Inside each bioreactor, two vertical PVC pipes (piezometers P1 and P2, both with 63 mm of diameter) were installed at 1.2 and 2.4 m from the water entrance. The pipes had holes at 26 cm from the bottom to let water flow inside. Each bioreactor was equipped with an effluent outlet system consisting of a PVC pipe 50 cm wide with drain

vales at different heights. This system is designed for upflow, where water is forced to move up into the bioreactor through the woodchip media before flowing out as effluent. The higher the drain valves are, the longer the HRT (Figure 7.1).

In addition, 18 net bags (28 x 28 cm with 2 x 2 mm sized mesh) were deployed in each bioreactor, each net bag with 200 g of dried (at 65 °C) woodchips. The net bags were placed at 20 cm and 70 cm from the bottom at 1 m, 2.4 m and 5 m from the bioreactor inlet to assess weight loss of woodchips over the duration of the experiment (Figure 7.1).

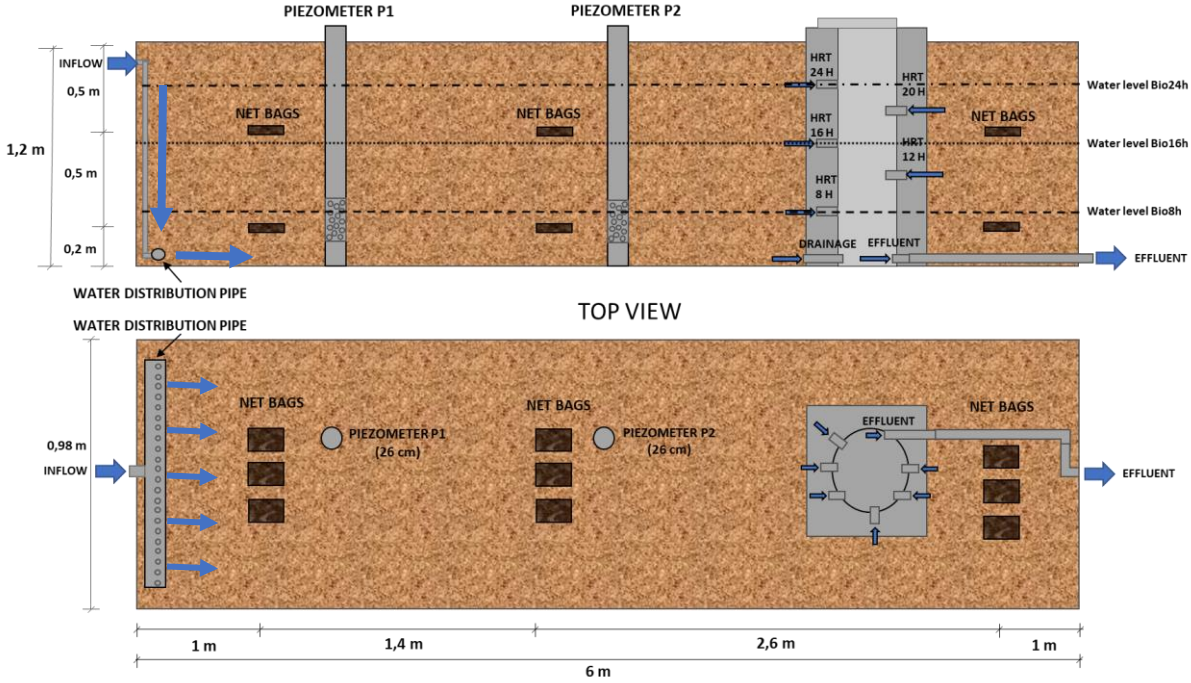


Figure 7.1. Lateral (above) and top (below) view of the woodchip denitrifying bioreactors. Positions of inflow, P1, P2, effluents, net bags. The outflow system with the drain valves at different heights is shown. Dashed lines represent the water levels of 8h (Bio8h), 16h (Bio16h) and 24 h (Bio24h) of hydraulic residence time (HRT).

### 7.2.2 Monitoring and sampling

During the first ≈ 20 days, the three bioreactors were subjected to an initial testing phase to calibrate the system flow working at 24 h HRT. During this phase, woodchips were washed for flushing the typical initial high dissolved organic carbon (DOC) contents that pose an environmental risk and do not represent the regular functioning regime (Schipper et al., 2010; Addy et al., 2016; Abusallout and Hua, 2017).

After that, bioreactors started working at 8 h (hereafter Bio8h), 16 h (hereafter Bio16h) and 24 h (hereafter Bio24h) HRT, respectively, in a continuous flow mode of 3 $m^3$ $d^{-1}$ per bioreactor. To check if the calculated HRT matched with the effective HRT, 25 kg of NaCl were dissolved in the bioreactor inflow distribution drum to increase salinity. The time until an increase in electrical conductivity (EC) occurred (i.e., the HRT) in the effluents was measured. The results of this test indicated that effective (or in situ) HRT was approximately the theoretical HRT for all three HRT selected for this experiment (8, 16 and 24 h) (Figure 7.2).

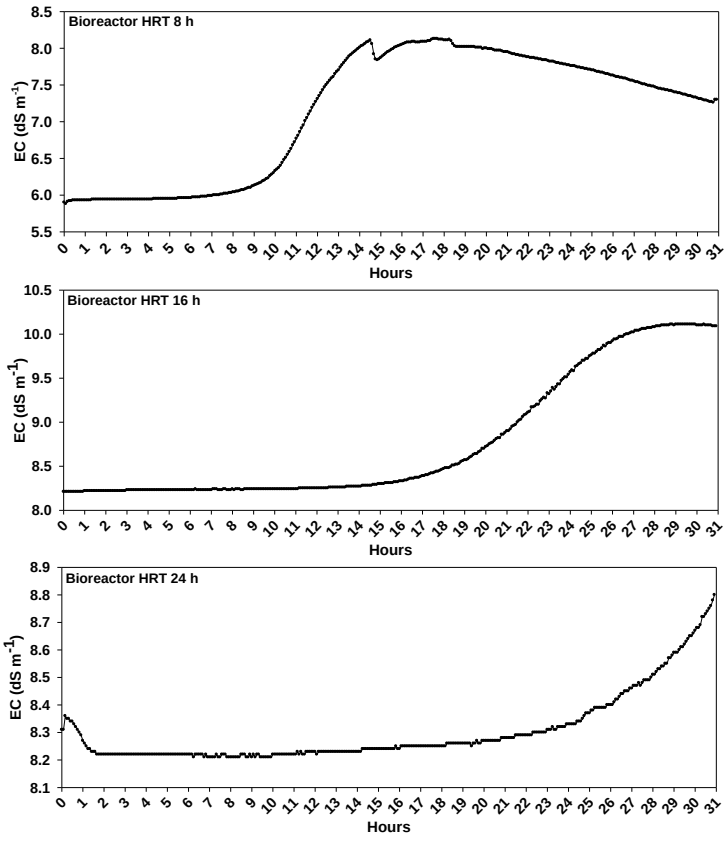


Figure 7.2. Period of salinity increase to check different hydraulic residence time (HRT) in each bioreactor. For each bioreactor, the effective HRT corresponds with the time in which EC started to increase.

A monitoring-sampling program was established according to the previous experience with bioreactors (Díaz-García et al., 2020; Maxwell et al., 2020a). From days 30 to 72 (weeks 4 to 9) bioreactors were monitored 3 days per week, two days a week between days 76 to 119 (weeks 10 to 16), and three days a week between days 125 to 566 (weeks 17 to 80).

Each sampling day, several parameters were measured *in situ* and water samples collected. Prior to taking *in situ* measurements and collecting samples, stagnated water inside the PVC pipes was removed using a polyethylene (PE) sampler, allowing the pipe refill with water in contact with woodchips. The pH, temperature, electrical conductivity (EC) and oxidation-reduction potential (ORP) were measured by placing a calibrated multiparameter instrument (Hanna HI 98194 pH/EC/DO Multiparameter) in the inflow distribution drum, in P1 and P2, and the effluent outlet system of each bioreactor (868 data points in total).

The ORP values were adjusted according to Vepraskas and Faulker (2001), by adding +200 mV to the measured values (the voltage of the Ag/AgCl reference electrode at 20 °C).

After measuring the aforementioned parameters, water samples were collected (100 mL) from the same four points previously named. Samples were filtered through Microsart CN-Filter filters, 0.45 µm pore size.

Three net bags (buried at 1 m, 2.4 m and 5 m from the inlet) (Figure 7.1) were extracted at weeks 25, 55 and 80 (6, 12 and 18 months respectively) after the experiment began. The woodchips were oven dried at 65°C until constant weight and weighed to assess woodchips degradation.

Nitrous oxide ($N_2O$), methane ($CH_4$), carbon dioxide ($CO_2$) and ammonia ($NH_3$) emissions were measured at nine points within each bioreactor in October 2020 to estimate rates of greenhouse (GHG) emissions at the three HRT. Concentrations were continuously detected by a photoacoustic spectroscopy multi-gas analyzer (GASERA ONE).

### 7.2.3 Water sample analyses and calculation of bioreactors performance

All the water samples collected were analyzed for $NO_3^-$, $NO_2^-$, $Cl^-$, $SO_4^{2-}$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ with a double channel chromatographic system 850 Professional Ion Chromatography Metrohm (868 samples in total). DOC was only analyzed one day a week, in the inflow water and in the effluents of the three bioreactors (237 samples in total), with a TOC-V CSH Shimadzu analyzer All of these analyses were performed in the Technological Research Support Service of the Technical University of Cartagena.

Sulfide ($H_2S$), ammonia ($NH_4^+$-N) and soluble reactive phosphorus (SRP) were measured in the bioreactor effluents between day 394 and 566 (the end of the experiment). These measurements were made with a V/UV Spectrometer, at λ = 670 mm for $H_2S$ (according to Cline, 1969) and $NH_4^+$ (according to Neiker, 2005), and λ = 825 mm for SRP (according to Murphy and Riley, 1962). Unfortunately, operational problems prevented the analysis of $H_2S$, $NH_4^+$ and SRP in samples collected before day 394.

In order to evaluate the performance of the bioreactors, nitrate removal efficiency (NRE) and nitrate removal rates ($R_{NO3}$) were calculated according to Christianson et al. (2015). (Eq. (1 and 2)):

$$\text{NRE}\ (\%) = \frac{(\text{NO}_3^- \text{–N influent concentration} - \text{NO}_3^- \text{–N effluent concentration})}{\text{NO}_3^- \text{–N influent concentration}}\ x\ 100 \quad (1)$$

$$\text{R}_{NO3}(\text{g N m}^{-3}\ \text{d}^{-1}) = \frac{(\ \text{NO}_3^- \text{–N influent concentration} - \text{NO}_3^- \text{–N effluent concentration})}{V_{saturated\ woddchips}\ \ x\ t} \quad (2)$$

Where influent concentration was the $NO_3^-$-N in the initial water (g N $m^{-3}$), effluent concentration was the $NO_3^-$-N in the effluent (g N $m^{-3}$), $V_{saturated\ woodchips}$ was the volume of saturated woodchips ($m^3$) and *t* was the HRT of each bioreactor (d).

## 7.3. Results

### 7.3.1 Temperature and physicochemical parameters

Inflow water temperature varied between a minimum of ≈13°C (January 14th) and a maximum of ≈ 27 °C (August 8th) with an average of 22.7 ± 0.7 °C (Table 7.1). Water temperature inside the bioreactors (Figure 7.3) reached maximum values of ≈ 25 - 27 °C in July-August 2019 and 2020, and minimum values of ≈ 13 - 15 °C in January-February 2019.

Table 7.1. Characteristics of inflow water in woodchip bioreactors. Values are the mean ± standard error. Average, minimum (Min.) and maximum (Max.) are referred to the complete study period (80 weeks). n = 78.

| Parameter | Average | Min. - Max. | Parameter | Average | Min. - Max. |
|---|---|---|---|---|---|
| **pH** | 7.6 ± 0.05 | 7.1 – 7.9 | **EC (mS cm$^{-1}$)** | 7.2 ± 0.1 | 5.7 – 8.5 |
| **ORP (mV)** | 369 ± 0.9 | 168 – 455 | **$NO_3^-$-N (mg L$^{-1}$)** | 33.9 ± 0.2 | 20.3 – 41.2 |
| **Temperature (°C)** | 22.7 ± 0.2 | 12.7 - 27.1 | **DOC (mg L$^{-1}$)** | 5.4 ± 0.2 | 2.5 – 15.6 |

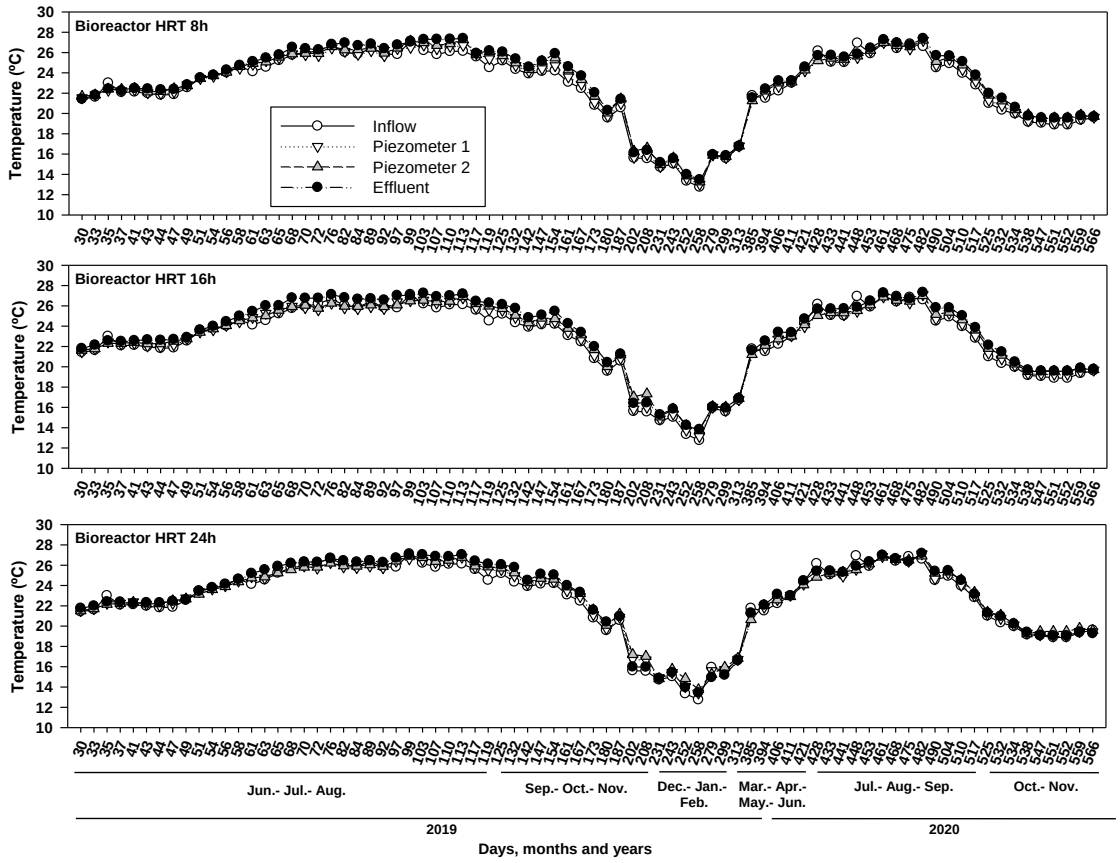


Figure 7.3. Temperature in three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

Since temperature was similar in P1 and P2 and in the effluents of the three bioreactors (Figure 7.3), the three values were averaged per sampling day to give an average daily temperature for the bioreactor.

The pH of the inflow water had negligible change during the study period, with inflow pH of 7.6 ± 0.05 (n = 78; Table 7.1). Inside the bioreactors, pH was similar at the three HRTs and tended to decrease from the inflow and piezometer P1 (average pH for the three bioreactors = 7.26 ± 0.007) to the effluent (average for the three bioreactors = 7.04 ± 0.01) (Figure 7.4).

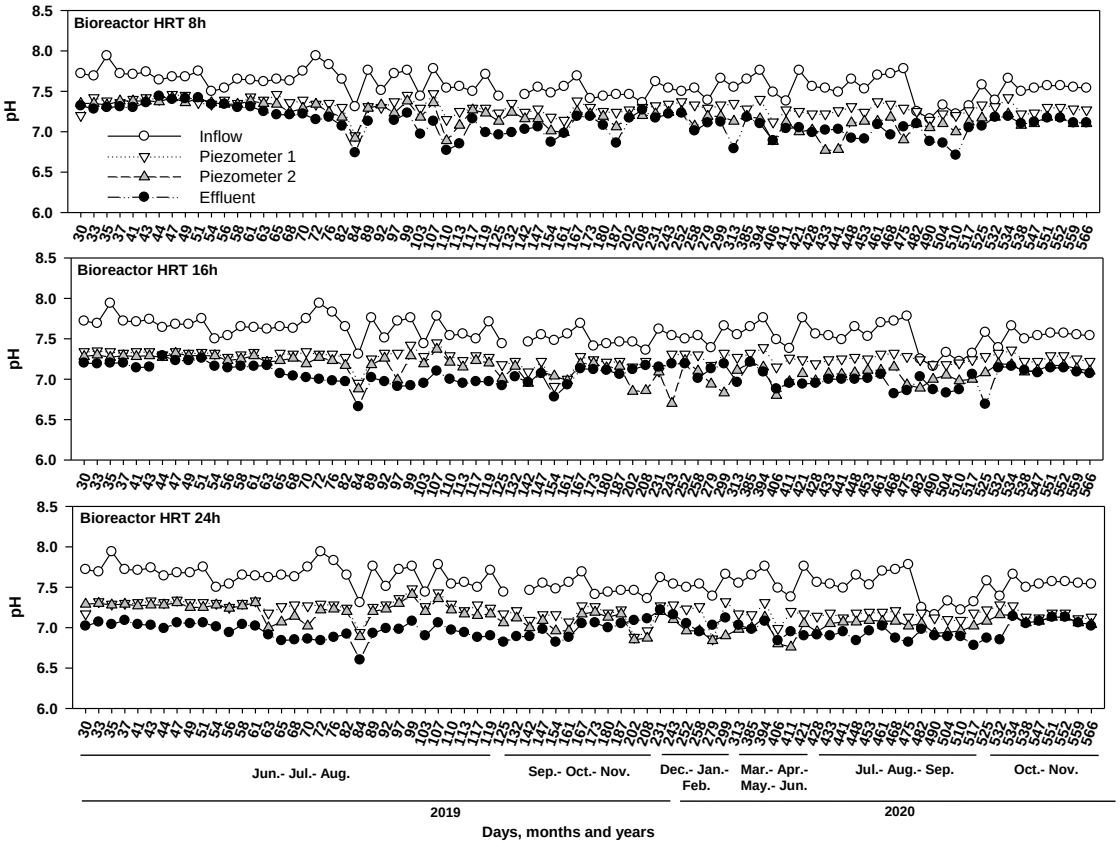


Figure 7.4. Values of pH in three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

The EC of the inflow water varied between 5700 and 8500 µS $cm^{-1}$ (7190 ± 3.2, average ± SE) and was relatively constant inside the three bioreactors (7248 ± 1.77 µS $cm^{-1}$) and in the effluents (7220 ± 1.8 µS $cm^{-1}$). The EC inside the bioreactor and at the effluent did not appear to be affected by position relative to the inflow or the HRT (Figure 7.5).

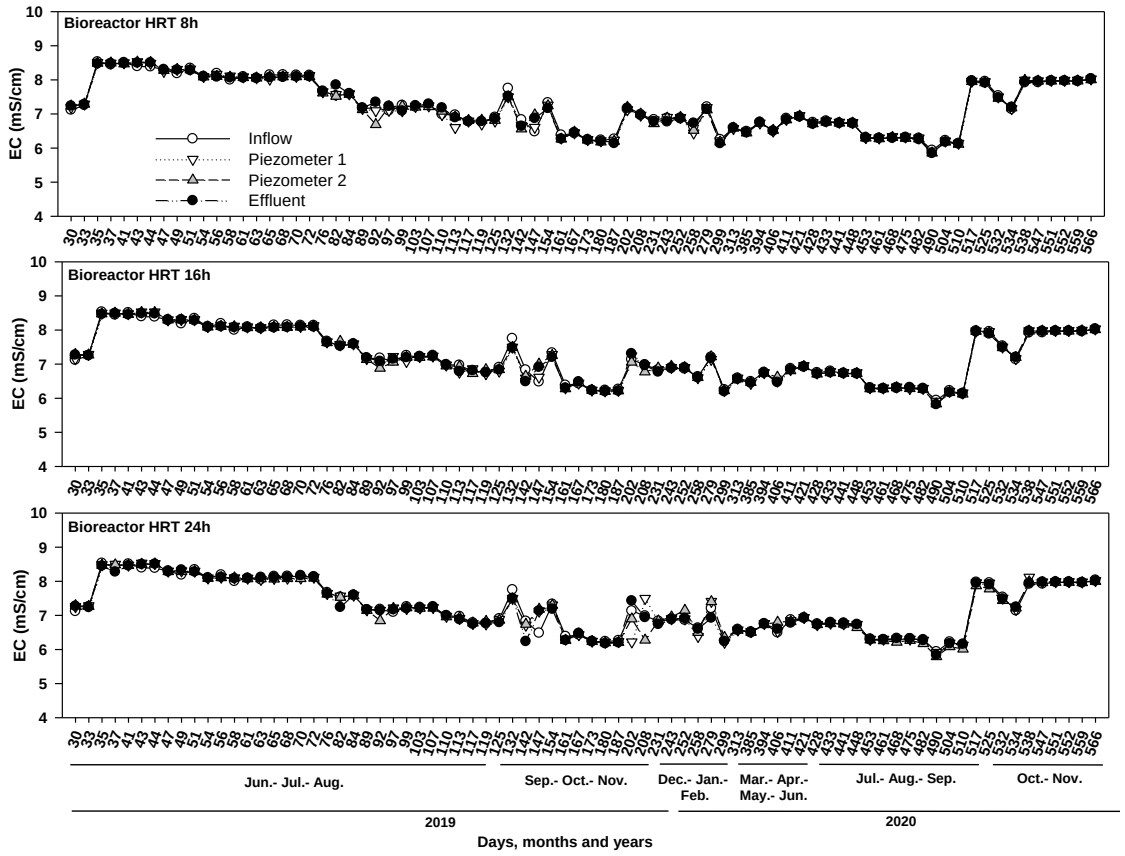


Figure 7.5. Values of EC in three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

The ORP in the inflow water varied between + 169 and + 455 mV, with an average value ≈ 368 mV (Table 7.1). In contrast with values of pH and EC, this parameter varied substantially inside bioreactors depending on the distance from the inflow (Figure 7.6) although with a similar rate among the three bioreactors. At 1 m from the inflow (P1) the ORP values showed little variation during the first 125 days (until end of August 2019) with values between ≈ 150 and ≈ 350 mV. From this date onwards the values were more variable, with decreases as low as ≈ - 100 mV and increases up to values ≈ 350 mV, mainly in the Bio24h bioreactor. In piezometer P2 (at 2.5 m from the inflow) the ORP values were similar to P1 during the first ≈ 60 days in Bio8h and Bio16h, but later the erratic behavior observed in P1 also occurred in P2, with strong ORP oscillations. This high variability in ORP values was observed throughout the experiment in P2 of Bio24h. Finally, ORP values in the outflow were always ≈-150 mV for all the three bioreactors.

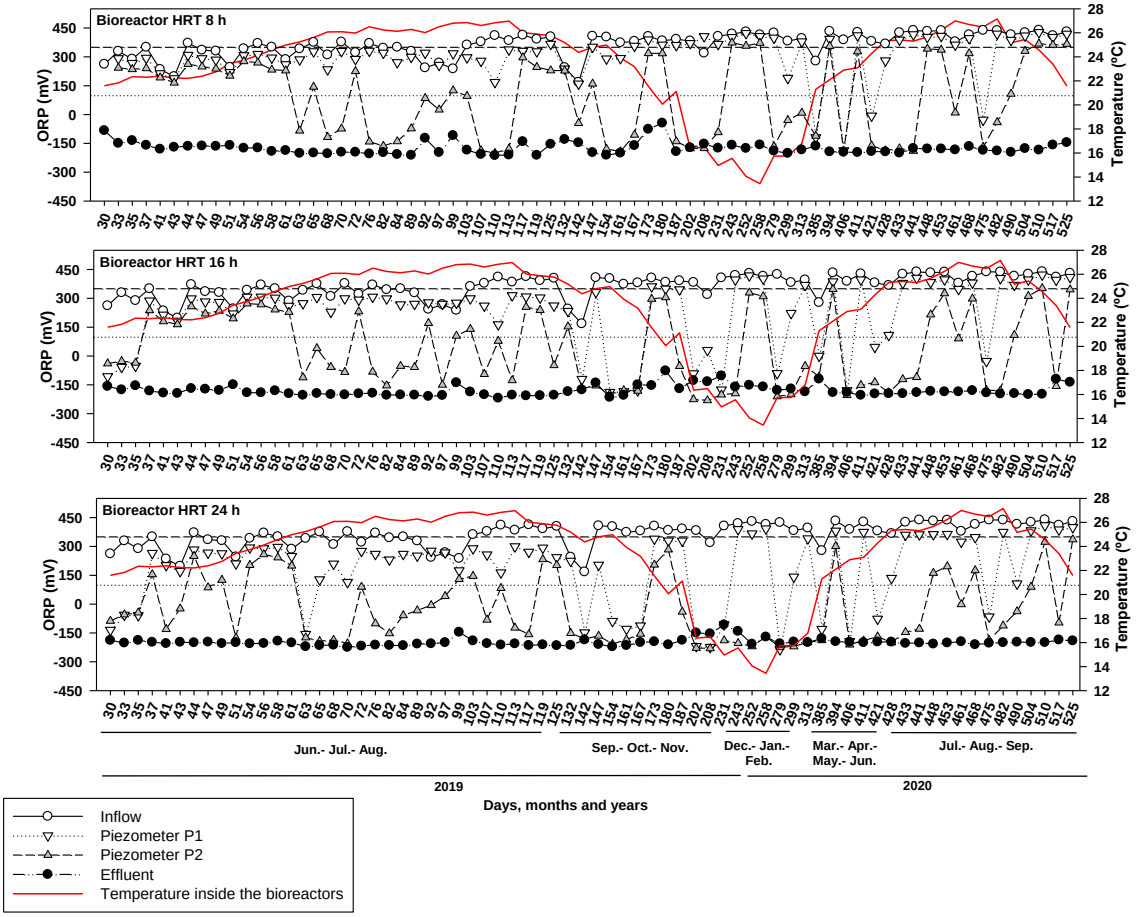


Figure 7.6. Oxidation Reduction Potential (ORP) in three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h. Average daily temperature is also shown. Horizontal dashed lines show the range for suboxic conditions, which is the optimal for denitrification (ORP between 100 and 350 mV) (Otero and Macias, 2003).

### 7.3.2 DOC concentrations and woodchips weight loss

The inflow water had low concentrations of DOC, with an average concentration during the study period ≈ 5.4 mg C $L^{-1}$ (Table 7.1). During the initial woodchips washing, effluent DOC concentrations reached ≈ 546 mg C $L^{-1}$ (average of the three bioreactors, day 1, first 24h) but dropped to ≈309 mg C $L^{-1}$ on day 3, decreasing to concentrations lower than 110 mg C $L^{-1}$ at day 15 and to ≈ 40 mg C $L^{-1}$ at day 22 (Figure 7.7).

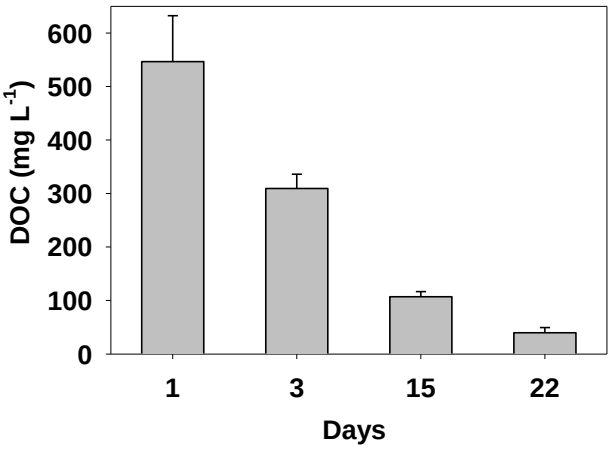


Figure 7.7. DOC concentrations during bioreactor start-up of woodchip washing, when the three bioreactors worked at 24 h of hydraulic residence time.

Once bioreactors started working at their regular operating mode (continuous flow at 8, 16 and 24 h HRT respectively), differences in DOC concentrations among bioreactors were observed (Figure 7.8). Between days ≈ 30 and ≈ 70, DOC concentration was higher in the Bio24h (17 ± 0.7 mg C $L^{-1}$) than at 8h (6.6 ± 0.5 mg C $L^{-1}$) and Bio16h (8.2 ± 0.6 mg C $L^{-1}$). However, from day 71 onwards the concentrations at the three bioreactors were similar (≈ 8 - 10 mg C $L^{-1}$), except for some sudden peaks at 8h or 16h HRT on days 113, 119, 243, 411, 428 and 433. Until day ≈ 450, DOC concentrations tended to increase when temperature increased, but from this date onwards changes in temperature did not appear to affect effluent DOC concentrations.

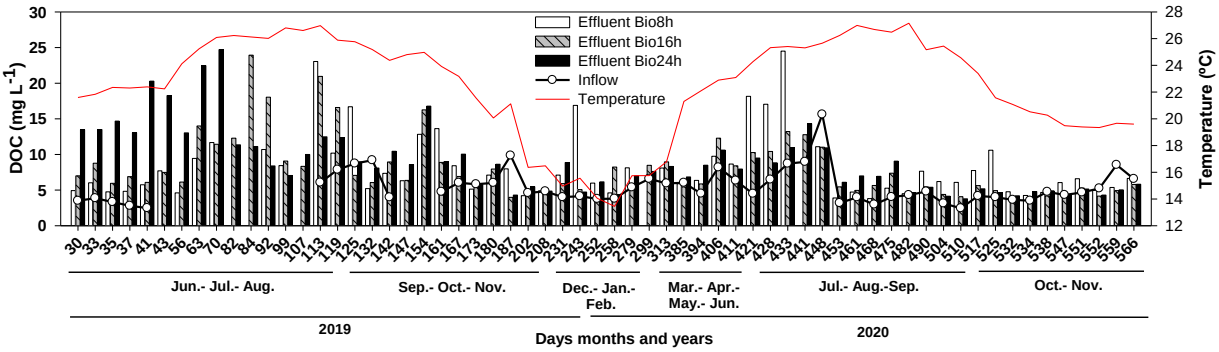


Figure 7.8. Concentrations of dissolved organic carbon (DOC) in inflow and in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h. Average of daily temperature inside the bioreactors is also shown.

Six months after starting bioreactor operation, net bags buried at 70 cm from the bottom (i.e., shallower) had lost ≈ 40 % of the initial weight (mass remaining ≈ 125 g) in Bio8h, 38 % (mass remaining ≈ 131 g) in Bio16h, and ≈ 16 % (mass remaining ≈ 170 g) in Bio24h (Figure 7.9). After 18 months, mass remaining was significantly lower than at 6 months in Bio8h (≈ 92 g), but not in Bio16h and Bio24h (≈ 104 and ≈ 161 g respectively). Mass remaining in Bio8h and Bio16h were significantly lower than in Bio24h at the three sampling times.

Not significant differences in mass remaining (≈80-90%; ≈170-180 g) were found among bioreactors for any sampling time in bags buried at 20 cm from the bottom (i.e., deeper) (Figure 7.9). A significant decrease in mass remaining between month 6 and month 18 was also observed in Bio8h.

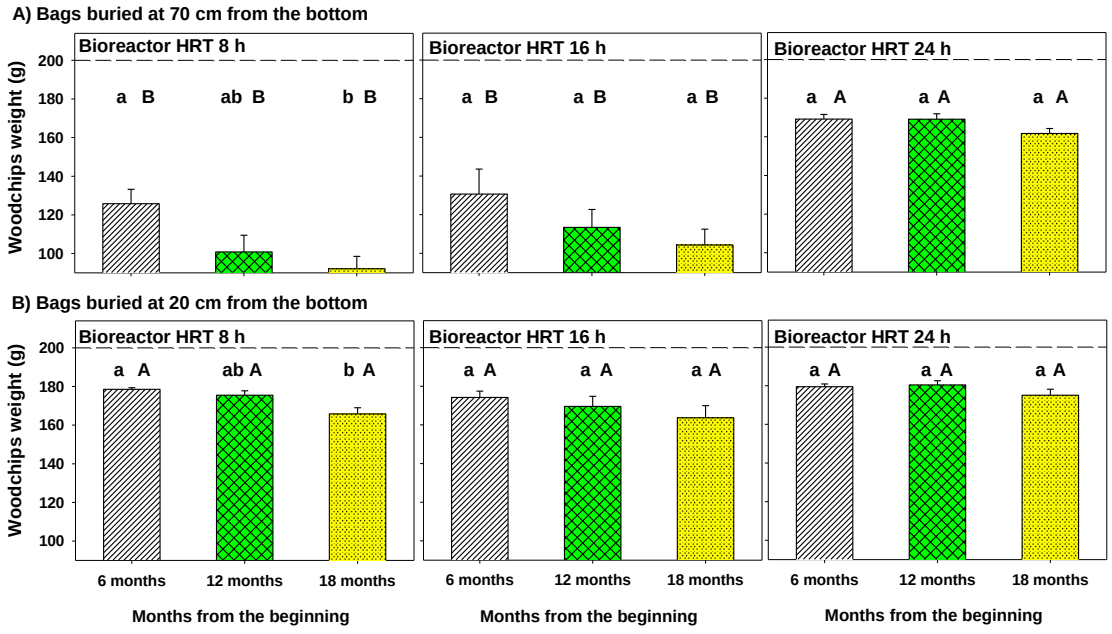


Figure 7.9. Weight loss of the woodchips inside net bags in the three bioreactors at the three sampling times. Dashed line indicates the initial weight (200 g). Bars represent average and lines above bars SE (n=3). Different small letters indicate differences among sampling times within a bioreactor and depth. Capital letters indicate differences among the three bioreactors for a given sampling time and depth. One-way ANOVA and Tukey post-hoc test ($p \leq 0.05$). HRT: Hydraulic Residence Time.

A visual indicator of woodchips degradation was the decrease in the woodchips level inside the three bioreactors between days 1 and 566. The levels had decreased 33 cm in Bio8h, 31 cm in Bio16h, and 17 cm in Bio24h (Figure 7.10 and 7.11).

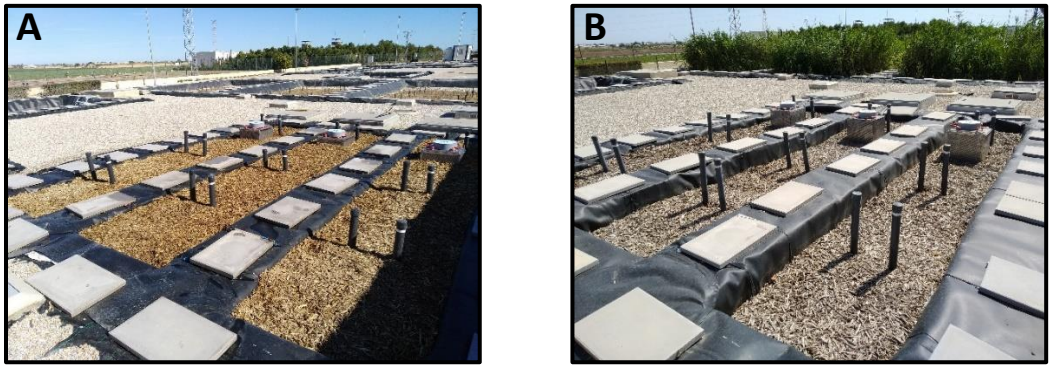


Figure 7.10. General view of woodchip levels in the bioreactors on day 1 (A) and on day 566 (B) of the experiment.


Figure 7.11. Detail of woodchips levels on day 566 of the experiment.

### 7.3.3. $NO_3^-$-N concentrations, $R_{NO3}$ and NRE

Inflow $NO_3^-$-N concentrations were between 20 and 40 mg $NO_3^-$-N $L^{-1}$ with an average (±standard error) of 34 ± 0.2 mg $NO_3^-$-N $L^{-1}$ throughout the 566 days of the experiment (Table 7.1, Figure 7.12). In general, for the three bioreactors, $NO_3^-$-N concentrations in the piezometer P1 were higher than in P2 and in the effluents, but data show that the degree to which $NO_3^-$ concentrations decreased along the bioreactor length was affected by the HRT, the time elapsed from the beginning of the experiment, and the temperature inside the bioreactors (Figure 7.12).

In Bio8h, between days 30 and 56 $NO_3^-$-N concentrations were relatively stable for P1 ≈ 34 mg $L^{-1}$, P2 ≈ 26 mg $NO_3^-$-N $L^{-1}$ and in the effluent ≈ 24 mg $NO_3^-$-N $L^{-1}$ (Figure 7.12). Between days ≈ 30 to ≈ 167 and ≈ 406 to ≈ 552 (both periods with temperature higher than ≈ 23 - 24 °C) $NO_3^-$-N concentrations in water from P2 and in the effluent were similar, and lower than in P1. However, between days ≈ 167 to ≈ 394, $NO_3^-$-N in P2 and in the effluent increased until being similar than in P1.

In Bio16h and Bio24h, $NO_3^-$-N concentrations were consistently lower in the effluents than in P1 and P2 throughout the study period, except between days ≈ 167 to ≈ 394, when temperature decreased to ≈ 15 - 20 °C (Figure 7.12). During this ≈ 227 days period, $NO_3^-$-N concentrations were highly variable and, on many occasions, similar in P1, P2 and in the effluent.

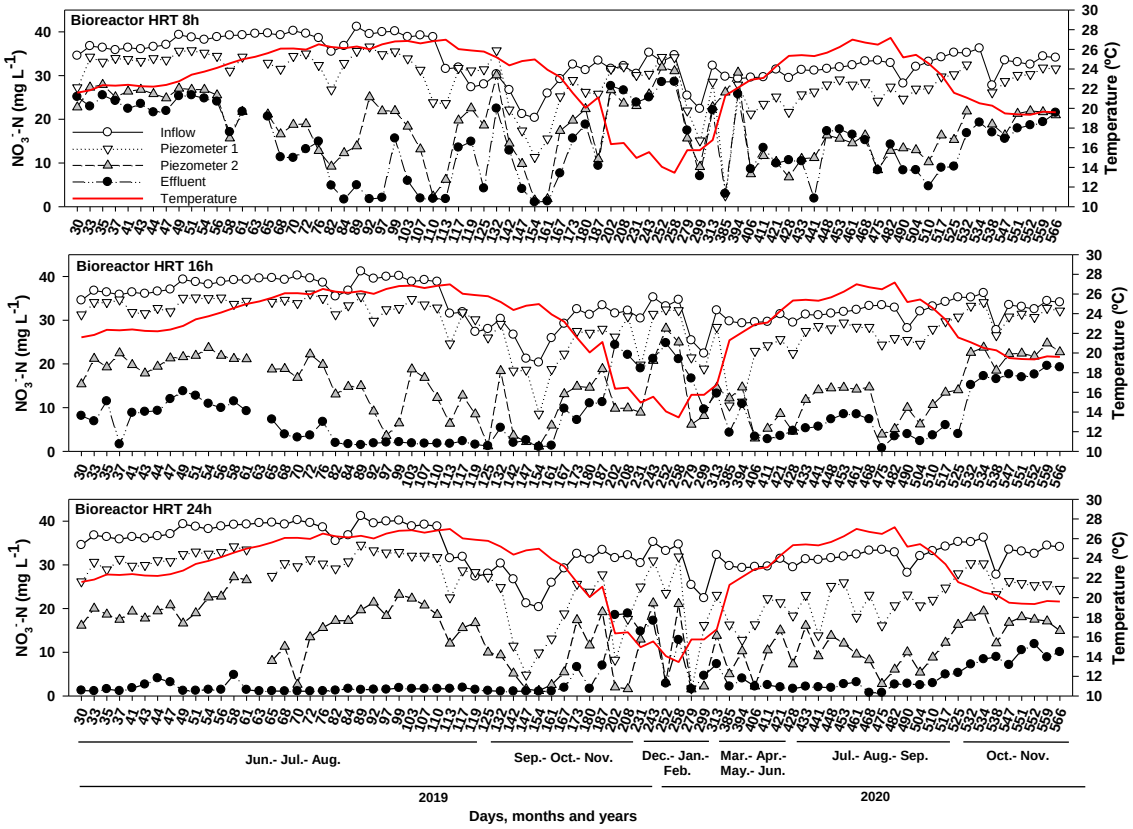


Figure 7.12. Daily average of nitrate ($NO_3^-$-N) concentration in the inflow, P1, P2 and in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h. Temperature inside the bioreactors is also shown.

The Nitrate Removal Rate ($R_{NO3}$) corresponded with changes in $NO_3^-$-N concentrations throughout the 566 days of the experiment (Figure 7.13). Average (±standard error) $R_{NO3}$ in the effluents over the study period were 8 ± 0.2 g N $m^{-3}$ $d^{-1}$ (Bio8h), 10.9 ± 0.2 g N $m^{-3}$ $d^{-1}$ (Bio16h), and 12.6 ± 0.2 g N $m^{-3}$ $d^{-1}$ (Bio24h). Between days ≈ 30 to ≈ 56, the $R_{NO3}$ in the effluents ranged between ≈ 5 to ≈ 15 g N $m^{-3}$ $d^{-1}$, and tended to increase between days ≈ 58 and ≈ 113, mainly at 8 h HRT (≈ 12 g N $m^{-3}$ $d^{-1}$). From days ≈ 117 to ≈ 258 $R_{NO3}$ tended to decrease (≈ 6, ≈ 8, ≈ 10 g N $m^{-3}$ $d^{-1}$, for Bio8h, Bio16h, and Bio24h, respectively) at the same time that temperature did, and between days ≈ 279 to ≈ 525 $R_{NO3}$ tended to increase again (≈ 8, ≈ 10, ≈ 11 g N $m^{-3}$ $d^{-1}$, for Bio8h, Bio16h, and Bio24h, respectively) coincidental with temperature increase. From day ≈ 532 onwards $R_{NO3}$ in the effluents tended to decrease to values below ≈ 10 g N $m^{-3}$ $d^{-1}$.

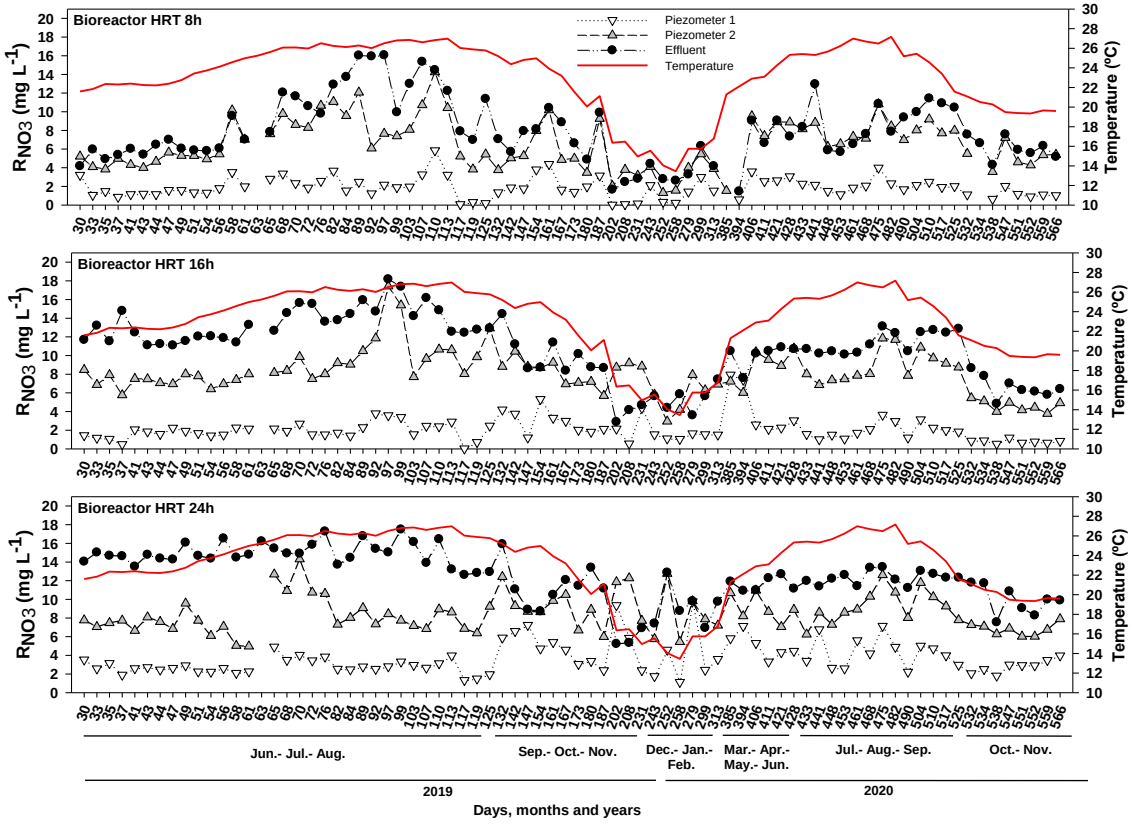


Figure 7.13. Nitrate Removal Rates ($R_{NO3}$) in P1, P2 and in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h. Temperature inside the bioreactors is also shown.

In general, the NRE increased when temperature and HRT were higher (Figure 7.14), mainly in Bio16h and Bio24h. Between days 30 to 82 (temperature between 21.6 and 26.5 °C), the NRE in Bio24h was ≈ 95 %, while in Bio16h and Bio8h were ≈ 79 % and ≈ 47 %, respectively. Between days 84 to 161, when the range of temperature was between 23.9 and 27.1 °C, NRE in Bio16h increased to values similar to those of Bio24h (≈ 94 - 95 %). In this period, Bio8h showed the highest nitrate removal performance over the entire study period, reaching NRE values ≈ 80 - 90 %. From days ≈ 167 to ≈ 258, when temperature decreased (14.0 – 23.2 °C) NRE dropped, reaching average ≈ 35 % in Bio8h (minimum ≈ 12 %), average ≈ 48 % in Bio16h (minimum ≈ 23 %) and average ≈ 69 % in Bio24h (minimum ≈ 41 %). The NRE in the Bio24h was maintained at ≈ 90 % between days ≈ 252 to ≈ 510 (temperature between 13.5 and 27.2 °C) to decrease at percentages ≈ 65 - 85 % from day ≈ 510 util the end, when temperature dropped again (19.4 - 24.6 °C). In Bio16h the NRE progressively increased until reaching percentages of ≈ 90 - 95 % on days ≈ 468 - 475, during a period of warmer temperatures (≈ 26.5 - 27 °C), to decrease again at ≈ 40 - 55 % when temperature decreased to ≈ 19 - 20 °C between days ≈ 532 - 552. Finally, the NRE in Bio8h varied between ≈ 35 and 74 % from day ≈ 400 until the end of the study period, without clear relationships with temperature changes.

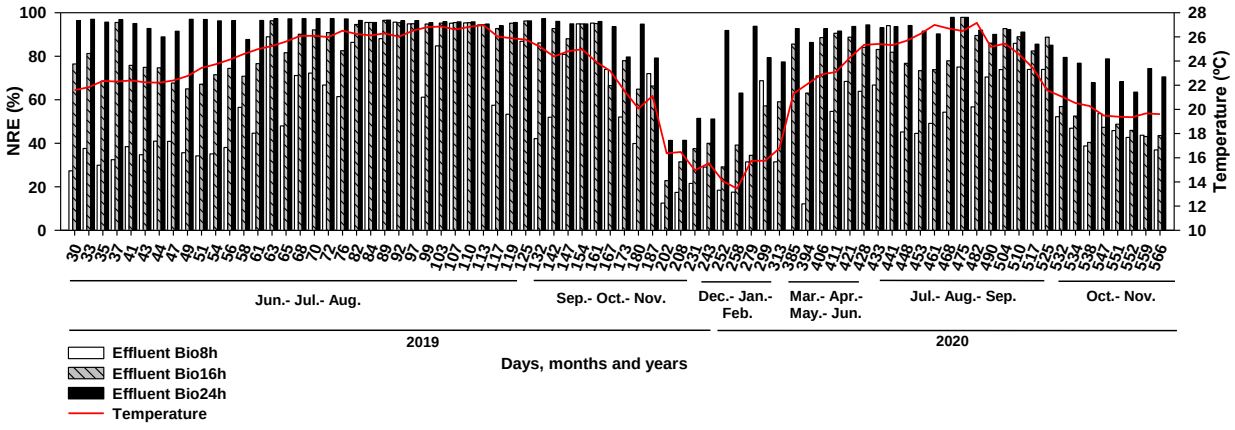


Figure 7.14. Nitrate Removal Efficiency (NRE) in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h. Temperature inside the bioreactors is also shown.

### 7.3.4. $H_2S$, $NH_4^+$-N and SRP in the inflow water and the effluents

The average concentrations of $H_2S$, $NH_4^+$-N and SRP in the inflow water (D7 drainage ditch) were < 0.09 mg $L^{-1}$. In the bioreactor effluents, $H_2S$ and $NH_4^+$-N concentrations were similar to or higher than concentrations in the inflow (Figures 7.15 and 7.16). Some peaks of higher than normal $H_2S$ concentrations occurred, mainly in Bio24h (e.g., day 299 ≈ 3 mg $L^{-1}$; day 406 ≈ 5 mg $L^{-1}$; day 475 ≈ 3.5 mg $L^{-1}$). Regarding $NH_4^+$-N, a peak of ≈ 7 - 8 mg $L^{-1}$ occurred on day 231, but concentrations were lower than ≈ 1 mg $L^{-1}$ from this day onwards. The concentrations of SRP (always lower than ≈ 2 mg $L^{-1}$) showed a different behavior than $H_2S$ and $NH_4^+$-N and were almost always lower than in the inflow water (e.g., days 510, 547, 551).

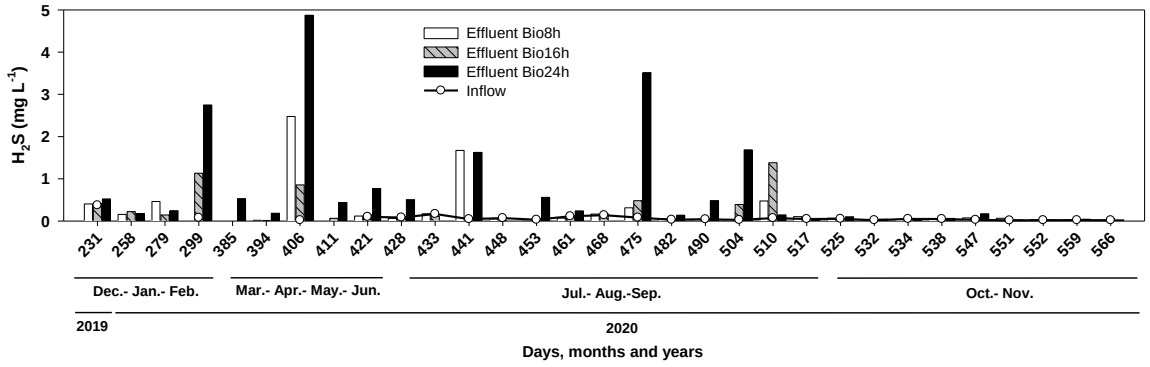


Figure 7.15. $H_2S$ in the in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

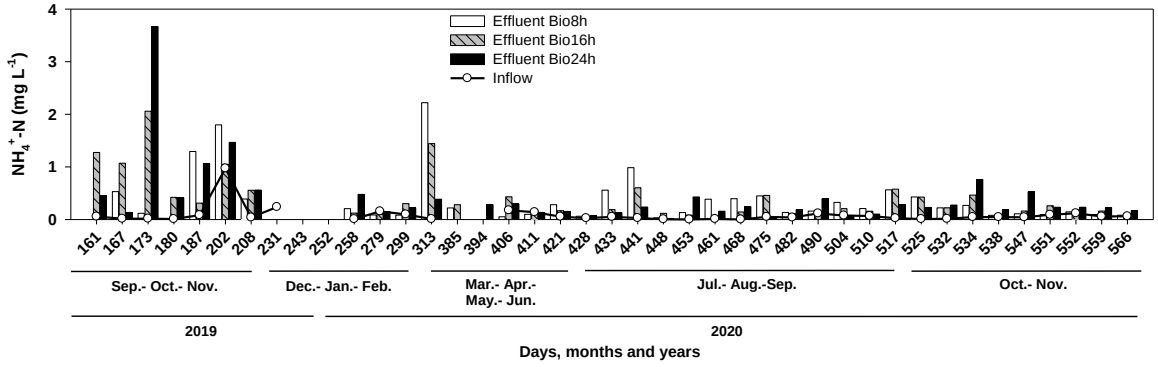


Figure 7.16. $NH_4^+$-N concentration in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

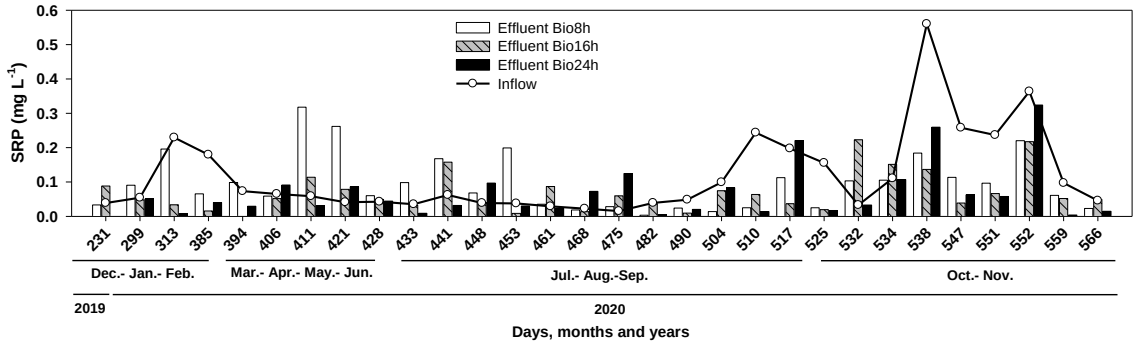


Figure 7.17. SRP in in the effluents of the three bioreactors with different hydraulic residence time (HRT): Bio8h, Bio16h and Bio24h.

### 7.3.5. GHG emissions

The $CO_2$ emissions tended to reach the highest values and were most variable in Bio8h (714 ± 127 mg $CO_2$ $m^{-2}$ $h^{-1}$; max = 1626; min = 190), and the lowest with less variability in Bio24h (504 ± 53 mg $CO_2$ $m^{-2}$ $h^{-1}$; max = 926; min = 232) (Figure 7.18). The $N_2O$ emissions were negligible in Bio8h and Bio16h (< 1.7 mg $N_2O$ $m^{-2}$ $h^{-1}$), but emissions reached high values with very high variability in Bio24h (41 ± 11 mg $N_2O$ $m^{-2}$ $h^{-1}$; max = 168; min = 3). Emissions of $CH_4$ and $NH_3$ were negligible (these two gasses were only detected on two occasions with concentrations < 0.5 mg $m^{-2}$ $h^{-1}$).

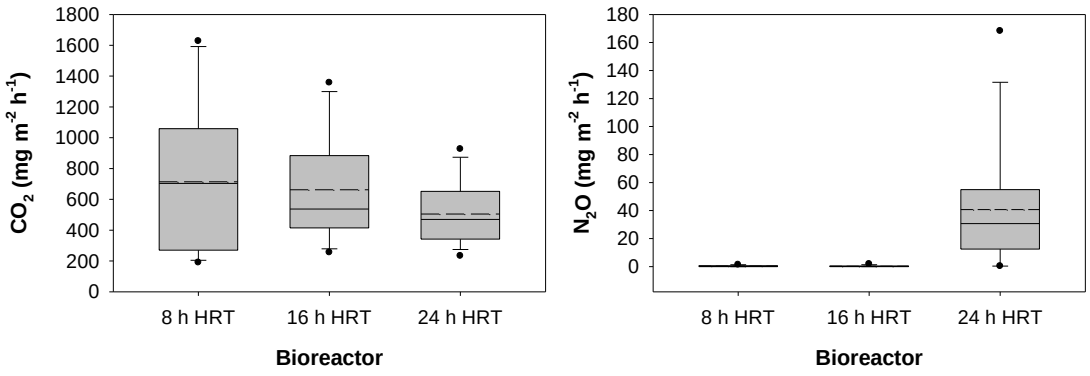


Figure 7.18. Box plots showing the greenhouse gas emissions measured inside three bioreactors (n = 15). HRT: Hydraulic Residence Time.

## 7.4. Discussion

### 7.4.1. Physicochemical conditions in the bioreactors

Microbial denitrification is a major pathway of nitrate removal in woodchip bioreactors, and this process is influenced by factors such as pH, salinity and ORP. The pH values in the three bioreactors remained in the suitable range for denitrification throughout the study period (pH ≈ 5.5 – 8) (Rivett et al., 2008; Albina et al., 2019), although changes were observed relative to the pH in the inflow water. In a flooded system, an increase of pH should be expected because a consumption of $H^+$ occurs under anaerobic respiration pathways (Stumm and Sulzberger, 1992), and because denitrification is a process that produces alkalinity (Reddy and Delaune, 2008). However, there was a trend of decreasing pH from the inlet to the outlet and effluent showed pH values ≈ 0.5 lower than the inflow. Other athors (e.g. Robertson and Merkley, 2009; Warneke et al., 2011) also found a drop of pH in woodchips biorectors. Several other processes favour $H^+$ production that might counterbalance the previously described processes which increase pH in flooded environments (Reddy and Delaune, 2008; Tercero et al., 2015). The water dissolution of the $CO_2$ released during anaerobic organic matter mineralization forms $H_2CO_3$, a weak acid that contributes to a decrease in pH. Organic acids produced as a result of the microbial activity may also contribute to drop the pH (Albina et al., 2019).

Salinity can have two different effects on microbial activity. On one hand, it might hinder microbial activity if the microbial population are not adapted, by direct toxicity or affecting osmotic balance (Lay et al., 2010). On the other hand, the presence of salts can facilitate breakdown of organic matter breakdown, and work to increase the amount of soluble organic compounds available for microbes (Craft, 2007; Weston et al., 2011; Marton et al., 2012; Steele and Aitkenhead-Peterson, 2013; Maxwell et al., 2020b). In this study, EC values were similar in the three bioreactors and did not change relative to the inflow. This indicates that salts were not being accumulated in the woodchips, which could be a factor that would negatively affect bioreactor performance and their useful lifespan.

The ORP is a key indicator of processes occurring in flooded systems, which can act as a surrogate parameter to understand microbial activity (Fiedler et al., 2007). When microorganisms use free oxygen for their metabolism in well-aerated environments, ORP values are > ≈ 350 mV (oxic conditions at pH ≈ 7, Vepraskas and Faulker, 2001; Otero and Macias, 2003; Reddy and Delaune, 2008; Unger et al., 2009). When a system is water saturated the ORP decreases in response to the drop in oxygen content. ORP values between ≈ 350 mV and ≈ 100 mV at pH ≈ 7 indicate that oxygen is depleted and other electron acceptors, such as nitrate, are used as electron acceptors during microbial metabolism via anaerobic pathways. When this occurs, nitrate is transformed into N gaseous forms ($N_2O$, $N_2$) by denitrification. ORP values < ≈ 100 mV indicate that sulphate ($SO_4^{2-}$) can be used as an electron acceptor leading to sulphide ($S^{2-}$) formation. In piezometer P1 and P2 of the three bioreactors, ORP values were almost always lower than ≈ 350 mV, indicating suitable conditions for denitrification. Furthermore, ORP was lower than ≈100 mV most of the time in P2, mainly in Bio24h, and in the effluents ORP values were extremely low (≈ - 150 mV), indicating potential risks for $S^{2-}$ formation, as discussed latter.

### 7.4.2. DOC concentrations in the effluents and woodchips degradation

Initial woodchip leaching showed extremely high DOC concentration that is characteristic of these systems at the beginning of operation. Previous assays for substrate selection had already showed the high DOC content that woodchips may release during early flushing (Díaz-García et al., 2020), as also observed by other authors (Healy et al., 2012; Malá et al., 2017). These extremely high DOC concentrations decreased greatly after the first weeks and did not increase again to similar levels during the rest of the study period, in agreement with other authors (Fenton et al., 2014; Malá et al., 2017). Since denitrifying bioreactors can have a usable lifetime of up to ≈10 years (Schipper et al., 2010; Fenton et al., 2014), the brief initial release of organic compounds is not considered a major drawback. Although the initial extremely high DOC concentrations in the effluents were not reached again during the 566 days of operation, unexpected peaks occurred at the three HRTs mainly during periods of high temperature (e.g., days 41, 63, 70, 113, 243, 428, 433).

Periodic increases in HRT due to possible malfunctioning of the system, for example by clogging of the bioreactor effluent pipes, could potentially lead to sporadic increases in DOC.

Regardless the extremely high DOC during woodchips washing and the unexpected peaks during regular working regime, baseline DOC concentrations were enough to support microbial activity, as shown by the drop in ORP and the reduction in $NO_3^-$-N concentrations. A tendency for DOC to increase during warmer periods and to decrease during colder periods was observed until day ≈ 453. This can be related with higher microbial activity when temperature increases and lower activity when temperature decreases (Robertson and Merkley, 2009; Hoover et al., 2015). From day ≈ 453 onwards DOC concentrations remained below ≈15 mg C $L^{-1}$, regardless of the temperature. This different behavior could be related to changes in DOC composition as the age of the woodchips increases (Moorman et al., 2010).

At the beginning of the experiment, fresh woodchips would have had a higher content of labile organic carbon (high cellulose content), which facilitates microbial respiration. However, when woodchips age increases, the quantity and quality of DOC provided decreases. The latter implies that microbials have to cope with more recalcitrant organic compounds more difficult to degrade such as tannin (Masbough et al., 2005; Maxwell et al., 2020b), as example, oak has a big quantity of tannin. The higher woodchip weight loss during the first six months in the three bioreactors (Figure 7.9) is further evidence of these more labile, easily degraded, organic compounds that were quickly consumed.

Differences observed in woodchips weigh loss among depths at which net bags were buried (Figure 7.9) deserve special attention. A much higher degradation was observed in the shallower bags (buried at 70 cm from the bioreactor bottom) of Bio8h and Bio16h than in Bio24h. In Bio8h and Bio16h the net bags were 50 and 13 cm above the water level, while in Bio24h the net bags were permanently underwater (see Figure 7.1). Hence, organic matter degradation of shallower bags must have occurred mainly via aerobic pathways in Bio8h and Bio16h, and via anaerobic pathways in Bio24h.

The higher efficiency of aerobic than anaerobic decomposition (Bridgham et al., 1998; Chow et al., 2006; Hansson et al., 2010; Moorman et al., 2010; Maxwell et al., 2018) explain the more intense degradation of woodchips buried above the water level than those underwater. In fact, differences in remaining woodchip mass between bioreactors were not observed in deeper net bags, which were always underwater.

Evolution of weight loss over time also supports this hypothesis of different decomposition pathways. In Bio8h, with a sheet of water, woodchip mass remaining in month 18 was significantly lower than in month 6. In this bioreactor differences were also observed even in the deeper bags. In Bio16h mass remaining had decreased at month 18, but not significantly, while in Bio24h mass remaining hardly changed.

### 7.4.3. Nitrate removal efficiency (NRE) and nitrate removal rates ($R_{NO3}$)

As previously explained, ranges of pH and ORP were suitable for denitrification, but the process can be also influenced by other factors including salinity, temperature and woodchip age (Robertson, 2010; Li et al., 2017; Ghane et al., 2018). NRE provides the percentage of $NO_3^-$-N removed and $R_{NO3}$ the quantity of $NO_3^-$-N eliminated per $m^{-3}$ $d^{-1}$.

In Section 7.4.1 it was discussed that salinity did not negatively affect microbial activity, and the high NRE and $R_{NO3}$ confirm that denitrification was not hindered by the salts. In general, Bio8h showed the lowest NRE and $R_{NO3}$ and Bio24h the highest, with Bio16h having an intermediate behavior. $R_{NO3}$ values fluctuated between ≈ 1.5 and ≈ 18 g N $m^{-3}d^{-1}$ depending on the HRT, with averages of ≈8 g N $m^{-3}d^{-1}$ forBio8h, ≈ 11 g N $m^{-3}d^{-1}$ for Bio16 h and ≈ 13 g N $m^{-3}d^{-1}$ for Bio24 h over the whole experiment. These values were higher than those found by Greenan et al. (2009), Warneke et al. (2011), Hoover et al. (2015) and Von Ahnen et al. (2016a), possibly due to the higher temperatures in our experiment.

When temperature increased up to ≈ 26 - 27 °C, maximum NRE (≈ 90 - 95 %) and $R_{NO3}$ (≈ 9 - 17 g N $m^{-3}$ $d^{-1}$) values were reached in the three bioreactors, regardless of the time elapsed since the beginning of the experiment (days ≈ 70 - 170 and days ≈ 440 - 525). When temperature dropped to ≈ 15 - 16 °C (days ≈ 202 - 279) the NRE reached minimum values of ≈ 12, ≈ 23 and ≈ 41 % in Bio8h, Bio16h and Bio24h respectively, and

$R_{NO3}$ also strongly decreased (≈ 2 - 9 g N $m^{-3}$ $d^{-1}$). A greater dependence of nitrate removal on temperature as woodchips age can be attributed to the lower quality of organic carbon remaining in the woodchips over time (Robertson, 2010; Xu et al., 2012). In our experiment, the three bioreactors showed high nitrate removal performance, and the effect of aging was not evident except in Bio8h, in which NRE and $R_{NO3}$ were clearly lower from day ≈ 394 onwards relative to the first year.

Other authors also found a strong relationship between nitrate removal and temperature (Addy et al., 2016; Hoover et al., 2016). Halaburka et al. (2017) stated that temperature explained 50 % of the variability in woodchip denitrification rates and Warneke et al. (2011) found an average of 7.63 g N $m^{-3}$ $d^{-1}$ when temperature varied between 15.5 and 23.7°C and 11.2 g N $m^{-3}$ $d^{-1}$ when temperature increased at 23.7 °C.

Although the ORP values in the bioreactors were suitable for denitrification, complete removal of nitrate from the influent was typically not achieved in Bio8h and Bio16h. This indicates that the HRT would have needed to be longer for the microorganisms to fully denitrify all nitrate present. When $NO_3^-$-N concentration is low, the necessary HRT can be short, but when $NO_3^-$-N load is high, more prolonged time is necessary for removing $NO_3^-$-N. The necessary HRT for full removal is impacted by temperature. At higher temperature microbial activity is enhanced and less time is needed for full nitrate removal. Christianson and Helmers (2011) found that HRT < 8 h was enough to achieve NRE≈ 60 % at temperatures between 3 and 15 °C, when inflow water contained between ≈7 and ≈13 mg $NO_3^-$-N $L^{-1}$. Greenan et al. (2009) needed almost 4 days to reach NRE ≈ 60 % at ≈10 °C of average temperature, with an inflow water containing 50 mg $NO_3^-$-N $L^{-1}$.

### 7.4.4. Potentially harmful substances and GHG emissions

Although bioreactors are considered a kind of ecological engineering option effective for nitrate removal, like other nature-based solutions they can have some environmental drawbacks. Occasional high DOC leaching and the presence of potentially harmful substances in the effluents and GHG emissions are among the most detrimental aspects (Grießmeier et al., 2019; Feyereisen et al., 2020).

Sulfate reduction to the $S^{2-}$ form occurs when most of the nitrates have been removed and microbes use sulfate ($SO_4^{2-}$) as the electron acceptor in their metabolism. This process is most can be more prevalent under certain environmental conditions (e.g., warm temperatures, high DOC contents, prolonged HRT). The increase of $S^{2-}$ concentrations in the effluents relative to the water inflow (Figure 7.15) indicated that the sulfate reduction process occurred in the bioreactors. This is consistent with the presence of $SO_4^{2-}$ in the inflow water and the extremely low ORP values that indicated suitable conditions for this process and with the anecdotal smell of rotten eggs (evidence of sulphidric acid, $H_2S$ production) in the bioreactor outlets (personal observation). Although most of the time $S^{2-}$ concentrations were low (< ≈ 0.1 mg $L^{-1}$), high concentrations were seen on certain days (≈ 1 – 5 mg $L^{-1}$), mainly in the Bio24h bioreactor. Dissolved $S^{2-}$ is toxic for biota (Reddy and Delaune, 2008; Rivett et al., 2008), and so the formation of this compound in bioreactors should be avoided (Christianson, 2011), particularly if the effluent is discharged to natural water bodies. Regular ORP monitoring and water sampling and analysis could be necessary to detect sulfide formation. If so, a reduction in the HRT could be applied or a drying phase forced to oxidize the formed $S^{2-}$. Reduced sulfur in bioreactor effluents could be managed using a complementary system with capacity to remove $S^{2-}$, such as a constructed wetland (Vymazal, 2014). A combination of both wetland and bioreator systems has been shown to have additional advantages for improving the performance and resilience of water treatment under shock loading events of other key contaminants such as TSS, BOD5 and TN (Sukias et al., 2018).

Similar to sulfide concentrations, $NH_4^+$-N concentrations in the effluents of the three bioreactors were higher than in the inflow, as also found by other authors (Lepine et al., 2015). In agreement with Greenan et al. (2009), $NH_4^+$-N concentrations in the effluents were <≈1 mg $NH_4^+$-N $L^{-1}$ most of the time. However, unexpected peaks reaching ≈1 to ≈4.5 mg $NH_4^+$-N $L^{-1}$ were found in the three bioreactors. The first step in organic nitrogen mineralization is the formation of $NH_4^+$ by ammonification (Reddy and DeLaune, 2008). Under anoxic conditions $NH_4^+$ is not transformed to $NO_3^-$ by nitrification, and its concentration in water increases. In addition, the dissimilatory $NO_3^-$ reduction to $NH_4^+$ (Lind et al., 2013; Bernard et al., 2015; Brin et al., 2015) could also contribute to

accumulation of $NH_4^+$, since this mechanism may occur at ORP values lower than 0 mV (Reddy and DeLaune, 2008).

Contrary to sulfide and $NH_4^+$-N, SRP decreased in the effluents relative to the concentrations in the water inflow (Figure 7.17), and a pattern depending on the HRT was not observed. Although bioreactors are known to be highly effective for nitrate removal, the role of these systems for P retention is not clear. Some studies have found certain capacity for SRP removal (Schipper et al., 2010) which could be favored by several processes, including; phosphorus consumption by microbials; P adsorption by extracellular polymeric substances produced by the microbial biofilm on the woodchips; and because some kinds of woodchips may also have the ability to adsorb phosphate to their surface (Hua et al., 2016). Also, P could be adsorbed onto mineral particles that are trapped within the woodchip media by gross filtration. The behavior of P inside the bioreactors and the capacity of these system for removing SRP deserve more attention in future research.

The main GHG emitted by hydric systems such as wetlands and bioreactors are carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$) (Elgood et al., 2010; Healy et al., 2012; Mander et al., 2014). The $CO_2$ emissions in this experiment were between ≈ 283 to ≈ 860 mg $m^{-2}$ $h^{-1}$ (Figure 7.18). These values are in the same range of concentrations found by Warneke et al. (2011) and Ghane et al. (2015), but were lower than the values reported in Woli et al. (2010). The tendency of greater emissions in Bio8h supports that organic matter mineralization was mainly via aerobic pathways, which matches with a higher woodchips weight loss due to a more effective microbial activity. $CH_4$ emissions were negligible, which differs from other authors who saw higher emissions, such as Elgood et al. (2010) with values between < 0.01 to 7.8 mg C $L^{-1}$ or Martin et al. (2019) with values between 1.5-1.7 g C $m^{-3}d^{-1}$. Davis et al. (2019) found a $CH_4$ rise when $NO_3^-$-N was below 10 mg $NO_3^-$-N $L^{-1}$. However, in our experiment, $CH_4$ emissions were not detected. The presence of high $SO_4^{2-}$ concentrations could hinder the ability of microbes to use $CO_2$ as an electron acceptor (and hence produce $CH_4$), since the first is a substrate that provides a better energetic efficiency (Reddy and de Laune, 2008).

The $NH_3$ emissions were not detected, which can contribute to explain the $NH_4^+$-N accumulation observed. Regarding $N_2O$ emissions, in Bio8h and Bio16h $N_2O$ concentrations were at similar ranges to those obtained by Elgood et al. (2010), Woli et al. (2010), Healy et al. (2012) and Christianson et al. (2013). However, emissions from Bio24h were higher in comparison with the cited studies.

## 7.5 Conclusion

The results showed that woodchip denitrifying bioreactors are a suitable option for the denitrification of saline effluents from intensive agriculture. Although extremely high DOC concentrations were seen immediately after bioreactor start-up, DOC decreased quickly within 30 days and concentrations were stabilized at relatively low concentrations that make the effluents should not cause environmetal drawbacks. Although effluent DOC is likely not a cause for concern, caution shoud be used because unexpected peaks of potentially harzardous high concentrations may occur under very high HRT.

Woodchips continously below the water level (i.e., under continuous anoxic conditions) suffered less degradation than woodchips above the water level. This finding can be useful to optimize the useful life span of the bioreactors, since woodchips above the water level will be more quickly degraded without an effective contribution to denitrification.

To optimize $NO_3^-$-N removal, HRT needs to be long enough for the microorganisms to perform denitrification even if physico chemical conditions are suitable to microbials perfirm the process. When $NO_3^-$-N concentration is low, HRT necessary for full nitrate removal can be short, but when $NO_3^-$-N load is high, more residence time can be necessary for removing enough nitrate. This is modulated by temperature, at higher temperature microbial activity is favored and denitrification needs shorter times, but when temperature decreases a longer period is required.

Emissions of $CO_2$ and $N_2O$, two GHG gasses, can be intense in the bioreactors. High quantities of woodchips above the water level favor $CO_2$ emissions, attributable to the predominance of aerobic metabolism pathways in microbial activity. The latter favors woodchip degradation and hence reduce bioreactors life span.

By contrast, $N_2O$ emissions are enhanced under strong anaerobic conditions. More research is necessary to improve these aspects of bioreactors management.

Special caution must be put due to the occasional discharge of potentially harmful compounds such as $S_2^-$ and $NH_4^+$. To mitigate this drawback a system for continuous monitoring should be implemented to allow modify bioreactor operation. Other option can be the installation of a constructed wetland for treatment of bioreactor effluents.

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# Chapter 8

# Conclusions

## 8.1 Conclusions

The general conclusion of the thesis is that citrus woodchip bioreactors are suitable systems to denitrify brine and other saline agricultural effluents with high nitrate load in the Campo de Cartagena, either under batch or continuous flow mode. Moreover, the mild temperatures in the study area allow better nitrate removal efficiency in reduced hydraulic residence time (24 hours or even less) than other places with colder climate.

This general statement is supported by the following specific findings:

1. Citrus woodchips were more favorable carbon media for denitrifying bioreactors than almond shell, chopped carob and olive bone, since they showed the highest nitrate reductions, the lowest organic carbon leaching, and had the lowest economic cost.
2. Salinity did not hinder denitrification in woodchip bioreactors.
3. Higher temperature and longer hydraulic residence time favored nitrate removal rate.
4. Woodchips aging negatively affected nitrate removal rate, particularly when temperature decreased below ≈ 20 ˚C. When sizing woodchip bioreactors, it must be considered that long-term declines in efficiency will be greatest at lower temperatures (< 20 °C).
5. Bioreactors drying phases increased nitrate removal in the subsequent flooding phases. This led to just after a drying phase the efficiency was less impaired by low temperature than after a period of continuous flooding. Hence, a way to optimize nitrate removal during colder periods is to increase the frequency of alternating drying-rewetting cycles.
6. While drying phases increased nitrate removal efficiency, they also increased woodchips degradation and, therefore, may shorten bioreactors life span.
7. During the first ≈ 3 - 4 weeks of bioreactors operation much caution must be put as extremely high carbon flushes occur. Hence, woodchips must be washed before bioreactors start operating and effluents must be properly managed to avoid environmental drawbacks. Furthermore, the nitrate removal efficiency

during this initial period does not represent the long-term performance of bioreactors.

8. Once finished the initial high organic carbon flush, concentrations within admissible levels for the environment are usual. However, unexpected high concentration peaks may occur during periods of high temperature or due to operation drawbacks inside bioreactors.
9. High concentrations of potentially harmful compounds for biota, such as sulfide, may occasionally occur during bioreactors life span. To try to avoid this, a continuous monitoring of the physicochemical conditions and water quality inside the bioreactors is necessary. However, since this management can be difficult to implement, additional measures such as routing bioreactor effluents to constructed wetlands to remove undesirable compounds other than nitrates may be a suitable strategy. If so, organic carbon peaks in the effluents would be also removed.
10. The role of woodchip bioreactors in phosphorus removal is not clear and therefore deserve further research.
11. Woodchip bioreactors were a source of $CO_2$ and $N_2O$ (greenhouse gasses, GHGs) to the atmosphere. $CO_2$ was mainly emitted when most of the woodchips were above the water level and $N_2O$ when most of the woodchips were underwater. Although some guidelines can be provided to try to reduce these emissions (e.g., optimization of hydraulic residence time), it must be assumed that they are much difficult to control in an effective way. Hence, the implementation of compensation measures for capturing GHG could be an option to balance the negative impacts of the emissions. In this sense, a constructed wetland, in addition to act as a buffer to treat bioreactor effluents, could contribute to capture $CO_2$ and to carbon storage.