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INFLUENCIA DE LA DIGESTIÓN ANAEROBIA CONVENCIONAL Y AVANZADA SOBRE LA COMPOSICIÓN Y FITOTOXICIDAD DEL BIOSÓLIDO: HACIA UN USO BENÉFICO

Tesis para optar al grado de Doctor en Ciencias Ambientales con Mención en Sistemas Acuáticos Continentales

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DEDICATORIA

A mi padre y a mi madre, por su amor y apoyo incondicional, por los sacrificios hechos para sacar a sus hijos adelante. Por enseñarme a vivir con honestidad y entrega. Por enseñarme que no hay nada más importante que la familia y el amor.

A mi hermana Verónica, por enseñarme que todo sacrificio tiene recompensa, que lo único que se necesita para salir adelante son ganas y trabajo duro, que de nada sirve el éxito si no se comparte con la familia.

A mi hermano Benjamín, por enseñarme que la humildad es una de las más grandes virtudes, que la mayor victoria se obtiene venciendo nuestros miedos.

A mi hermana Marta, por enseñarme a buscar siempre la excelencia y que lo más importante es hacer lo que nos gusta.

A mi familia y amigos, que fortalecieron mis alas y me impulsaron a volar tras mis sueños.

"He aprendido a mirar de una manera más viva:
como si mis abuelos por mi sangre miraran;
como si los futuros habitantes
alzaran mis pestañas.

Yo no miro la piel sino lo que en la piel
es fuego y esperanza.

Lo que aún en los muertos
sigue nutriendo razas.

Lo que es vida y es sangre
tras la inmovilidad de las estatuas."

Jorge Debravo

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RESUMEN

El objetivo de este proyecto fue evaluar la influencia de la digestión anaerobia convencional (DAC) y la digestión anaerobia avanzada (DAA) de lodos sanitarios en la composición (es decir en las características fisicoquímicas y microbiológicas, y en la concentración de microcontaminantes) y en la fitotoxicidad directa e indirecta de los biosólidos (BS) generados. La hipótesis de investigación fue que la DAA produciría un cambio más favorable en la composición y la fitotoxicidad de los BS en comparación con los generados por DAC, considerando como finalidad la aplicación benéfica de los BS.

Se operaron por aproximadamente 200 días, dos reactores anaerobios en condiciones mesofílicas a escala de laboratorio operados con un tiempo de retención de lodos de 30 días. Uno en fue alimentado directamente con lodos sanitarios (DAC) y el otro fue alimentado con el mismo lodo sanitario pero pre-tratado (DAA). Se utilizó un pre-tratamiento secuencial de ultrasonido seguido de una etapa térmica a baja temperatura (55°C). Durante el periodo de operación el desempeño de ambos reactores fue evaluado mediante la determinación de la reducción sólidos totales (ST), materia orgánica estimada mediante la demanda química de oxígeno (DQO) y el rendimiento de generación de metano.

Los BS generados por ambos reactores fueron destinados para diferentes propósitos. Una fracción fue utilizada para realizar caracterizaciones fisicoquímicas como determinación de ST, de sólidos volátiles (SV), pH, conductividad, humedad, contenido de metales, contenido de nutrientes (nitrógeno, fósforo y potasio), materia orgánica, entre otros. A otra fracción se le determinaron agentes patógenos como coliformes totales y fecales. Otra fracción de los BS fue destinada a los ensayos de fitotoxicidad directa e indirecta. Una última fracción fue utilizada en la determinación de los microcontaminantes (tonalide, galaxolide, triclosán, hidroxitolueno butilado, fenantreno, pireno, 4-nonilfenol, tert-octilfenol, bisfenol A y 17β-etinil estradiol).

El desempeño de la digestión anaerobia mejoró con la incorporación del pre-tratamiento secuencial. Con remociones de DQO y SV un 12.89% y 4.25% mayores que el sistema de DAC. El rendimiento de metano fue un 30.24% mayor en DAA que el sistema DAC. Respecto a las características fisicoquímicas de los BS, la concentración de nitrógeno total, fósforo total, amonio,

cobre, zinc y mercurio; fueron entre 1.05-1.58 veces mayores en los BS de DAA en comparación con los procedentes de DAC. Comportamiento relacionado principalmente con la solubilización de la materia orgánica debido al pre-tratamiento.

Considerando los 10 microcontaminantes determinados, ambos sistemas resultaron en la acumulación de 8 de los microcontaminantes. Siendo el triclosán el único compuesto removido durante la DAC y DAA y el 17β-etinil estradiol el compuesto que no se detectó ni en los lodos sanitarios parentales, ni en los BS. Sin embargo, la DAA resultó en la disminución de 7 de los 9 compuestos detectados respecto a la DAC.

La fitotoxicidad directa e indirecta de los BS y de los lodos sanitarios parentales, fue evaluada mediante dos plantas dicotiledóneas y una monocotiledónea, *Lactuca sativa, Raphanus sativus* y *Triticum aestivum*, respectivamente. Los resultados mostraron la alta fitotoxicidad de los lodos sanitarios, relacionada con la materia orgánica putrescible, es decir, la falta de estabilización. La fitotoxicidad indirecta de los BS de DAC causaron efectos positivos en *R. sativus* y *T. aestivum*, pero para *L. sativa* se observó una alta toxicidad. En el caso de los BS de DAA, estos mostraron un efecto benéfico o la no presencia de sustancias fitotóxicas para las tres plantas testeadas. En el caso de la fitotoxicidad directa los BS de DAC fueron ligeramente más benéficos que los BS de DAA, con aumentos entre 4-20% en el índice de germinación de DAC en comparación a DAA y con EC_{50} promedio de 164 g/kg y 159 g/kg, para los BS de DAC y DAA respectivamente.

Por tanto, se rechaza la hipótesis planteada. Aunque la aplicación del pre-tratamiento secuencial de ultrasonido seguido de un tratamiento térmico a baja temperatura (55°C) mejoró la conversión de la materia orgánica, incrementó la producción de metano, aumentó la remoción de organismos patógenos y de microcontaminantes. Los resultados de fitotoxicidad directa e indirecta de los biosólidos procedentes de digestión anaerobia convencional y avanzada, no mostraron diferencias significativas.

ABSTRACT

The aim of this project was to evaluate the influence of conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) of sewage sludge in the composition (i.e. physicochemical and microbiological characteristics, and the concentration of micropollutants) and in the direct and indirect phytotoxicity of the biosolids (BS) generated. The hypothesis was that the ADF produced more favorable changes in the composition and phytotoxicity of the BS than those generated by CAD, considering as a final purpose the beneficial application of the BS.

For 200 days, two laboratory-scale anaerobic reactors under mesophilic conditions were operated at sludge retention time of 30 days. One was fed directly with sewage sludge (CAD) and the other one was fed with the same sewage sludge but pre-treated (AAD). A sequential pre-treatment of ultrasound followed by a thermal low temperature (55°C) stage was used. During the operation time the performance of both reactors was evaluated by determining the total solid (TS) reduction, organic matter estimated as chemical oxygen demand (COD) and the methane yield.

The BS generated by both reactors were used for different purposes. A fraction was used to perform physicochemical characterizations such as determination of TS, volatile solids (VS), pH, conductivity, humidity, metal content, nutrient content (nitrogen, phosphorus and potassium), organic matter, among others. Another fraction was used to determined pathogens as total and fecal coliforms. Another fraction of the BS was used for direct and indirect phytotoxicity assay. A final fraction was used in the determination of microcontaminants (tonalide, galaxolide, triclosan, butylated hydroxytoluene, phenanthrene, pyrene, 4-nonylphenol, tert-octylphenol, bisphenol A and 17β-ethinyl estradiol).

The anaerobic digestion performance improved with the incorporation of the sequential pre-treatment of ultrasound followed by a low-temperature thermal stage. With COD and VS removals 12.89% and 4.25% higher than the CAD system. And the methane yield in AAD was 30.24% higher than the CAD system. Regarding the physicochemical characteristics of the BS, the concentration of total nitrogen, total phosphorus, ammonium, copper, zinc, and mercury; were between 1.05-1.58

times higher in the BS from AAD compared to those from CAD. Behavior related mainly to the solubilization of organic matter due to pre-treatment.

Considering the 10 microcontaminants determined, both systems resulted in the accumulation of 8 of the microcontaminants. Triclosan being the only compound removed during CAD and AAD, and 17β-ethinyl estradiol the compound that was not detected in either SS or BS. However, AAD resulted in a decrease of 7 of the 9 compounds detected with respect to CAD.

The direct and indirect phytotoxicity of the BS and the parental SS, which was evaluated by two dicotyledonous plants and one monocotyledonous; *Lactuca sativa*, *Raphanus sativus*, and *Triticum aestivum*, respectively. The results showed the high phytotoxicity of sewage sludge, related to putrescible organic matter, that is, the lack of stabilization. Indirect phytotoxicity of BS from CAD caused positive effects in *R. sativus* and *T. aestivum*, but for *L. sativa* high toxicity was observed. In the case of the BS from AAD, these showed a beneficial effect or the absence of phytotoxic substances for the three plants tested. In the case of direct phytotoxicity, the BS from CAD was slightly more beneficial than those from AAD, with increases between 4-20% in the germination index of CAD compared to AAD and with an average EC_{50} of 164 g/kg and 159 g/kg, for the BS from CAD and AAD, respectively.

Therefore, the hypothesis is rejected. Although the application of sequential pre-treatment of ultrasound followed by a low temperature treatment (55°C) improved the conversion of organic matter, the methane production increased, the elimination of pathogens and microcontaminants increased. The results of the direct and indirect phytotoxicity of the biosolids from conventional and advanced anaerobic digestion, showed no significant differences.

CAPÍTULO I

INTRODUCCIÓN

1. Introducción

Los asentamientos humanos y las actividades que en ellos se desarrollan producen una presión sobre los sistemas acuáticos continentales por la demanda de agua (uso y consumo) y la disposición de las aguas residuales, es decir, los efluentes que resultan del uso del agua en las viviendas (aguas servidas, AS), el comercio y la industria. La descarga de aguas servidas sin un tratamiento adecuado afecta el funcionamiento de los sistemas acuáticos donde son vertidas por los aportes de materia orgánica, nutrientes y compuestos químicos que producen variaciones en el oxígeno disuelto, posible eutrofización o toxicidad. La inadecuada gestión de las aguas residuales repercute tanto en la salud de los ecosistemas como en el bienestar de las personas. Por ejemplo, consecuencia de la insalubridad del agua de consumo y un saneamiento deficiente, en países de bajos y de medianos ingresos mueren más de 842 000 personas cada año, de las cuales cerca del 33% de las muertes está relaciona directamente con un saneamiento deficiente (WHO, 2018). Por ello, las plantas de tratamiento de aguas servidas (PTAS) cumplen un papel fundamental en la depuración adecuada de las aguas servidas para mantener la salud de los ecosistemas y del ser humano.

El sistema de lodos activados es una de las tecnologías ampliamente utilizada para el tratamiento de las AS, por ejemplo, en Chile más del 60% de las plantas de tratamiento de agua servida (PTAS) corresponden a esta tecnología. En este tipo de PTAS alrededor del 60% de la materia orgánica presente en el AS será transferida a los lodos sanitarios (LS), el manejo de estos implica un costo entre 30-65% del costo de operación de la PTAS (Pérez-Elvira et al., 2006). La disposición de los LS sin estabilización en rellenos sanitarios disminuye la vida útil del predio y podría generar problemas de emisión de gases de efecto invernadero y lixiviados (Du, 2015). Entre las tecnologías de tratamiento de LS se incluyen secado térmico, aplicación de cal, compostaje, incineración y digestión anaerobia. Luego de ser estabilizados pueden ser dispuestos en rellenos sanitarios, mono-rellenos, aplicados como mejorador de predios agrícolas o en la recuperación de suelos degradados.

La estabilización de LS mediante digestión anaerobia permite una reducción del volumen de lodo, tiene requerimientos de energía relativamente bajos, produce biogás que puede ser aprovechado, y genera un LS estabilizado (biosólido, BS) que podría tener un uso benéfico (McNamara et al., 2012; Stasinakis, 2012; Tiwary et al., 2015). Sin embargo, la digestión anaerobia convencional (DAC) de LS se ve limitada por la conversión de proteínas a aminoácidos, de carbohidratos a azúcares y de lípidos a ácidos grasos, es decir, la hidrólisis de los sólidos orgánicos. Por ello, se han implementado sistemas con digestión anaerobia avanzada (digestión anaerobia con un pre-tratamiento, DAA) para facilitar la hidrólisis del lodo y así mejorar el desempeño de la digestión anaerobia. El efecto de los pre-tratamientos en el desempeño de la digestión anaerobia ha sido estudiado principalmente con la finalidad de aumentar la producción de biogás y reducir el volumen de sólidos. No obstante, existe un vacío de conocimiento sobre la calidad del biosólido, tema fundamental para fomentar su uso benéfico o idear nuevas estrategias de tratamiento.

Si bien, el uso de BS presenta múltiples beneficios económicos y ambientales como recuperación de suelos deteriorados, disminución del uso de fertilizantes artificiales en predios agrícolas y reutilización de residuos. Sin embargo, su aplicación en suelo enfrenta un gran desafío: la seguridad alimentaria y ecosistémica debido a algunos de los componentes de los biosólidos. Lo que hace necesaria una evaluación integral de la calidad (física, química, microbiológica y fitotoxicológica) del biosólido. Por ello, el objetivo de este proyecto fue evaluar la influencia de la digestión anaerobia convencional y avanzada en la composición y fitotoxicidad del biosólido producido.

2. Sumario y estructura de la tesis

Esta tesis tiene como objetivo evaluar la influencia de la digestión anaerobia convencional y avanzada, en la composición (características fisicoquímicas, microbiológicas y en la concentración de microcontaminantes) y fitotoxicidad del biosólido obtenido. Evaluar la calidad del biosólido considerando parámetros fisicoquímicos, microbiológicos y fitotoxicológicos, impulsa el desarrollo de herramientas de evaluación ambiental, la optimización de los procesos de tratamiento de aguas servidas y de lodos sanitarios, así como el fomento a su gestión adecuada. Aspectos fundamentales para lograr un cambio de paradigma de “tratamiento para disponer” por “tratamiento para reusar”.

La incorporación del pre-tratamiento secuencial que integra procesos físicos y biológicos para promover la etapa hidrolítica de la digestión anaerobia mejora el desempeño de esta y a la vez genere un biosólido con un grado de estabilización mayor lo que podría conllevar a un mayor potencial de uso como remediador o fertilizante. Para confirmar esta hipótesis se desarrolló la presente investigación resumida en la Figura 1 y estructurada de la siguiente manera. En el Capítulo II se presenta el marco teórico, abordando y profundizando en conceptos de importancia sobre el tratamientos de las aguas servidas, los fundamento teóricos de la digestión anaerobia y los tipos de pre-tratamientos existentes. En el Capítulo III se establece la hipótesis del estudio, así como el objetivo general y los objeticos específicos.

Los Capítulos entre el IV y el VII se da respuesta a los objetivos específicos planteados de la siguiente manera. Los Capítulo IV y V responden el objetivo específico 1 y 2, mostrando los principales resultados del efecto de la digestión anaerobia convencional y avanzada en la concentración de microcontaminantes, así como una revisión bibliográfica sobre la presencia y el destino de los microcontaminantes durante la digestión anaerobia y su implicancia en el marco de la economía circular. Los Capítulos VI y VII se presentan los resultados relacionados con la fitotoxicidad directa e indirecta, respondiendo al objetivo específico 1 y 3.

Finalmente, en el Capítulo VIII se presenta la discusión general de los principales resultados de la tesis. El Capítulo IX presenta las principales conclusiones y las recomendaciones sugeridas. Terminando con los Anexos I y II donde se presentan los productos de la tesis (congresos nacionales e internacionales y las publicaciones).

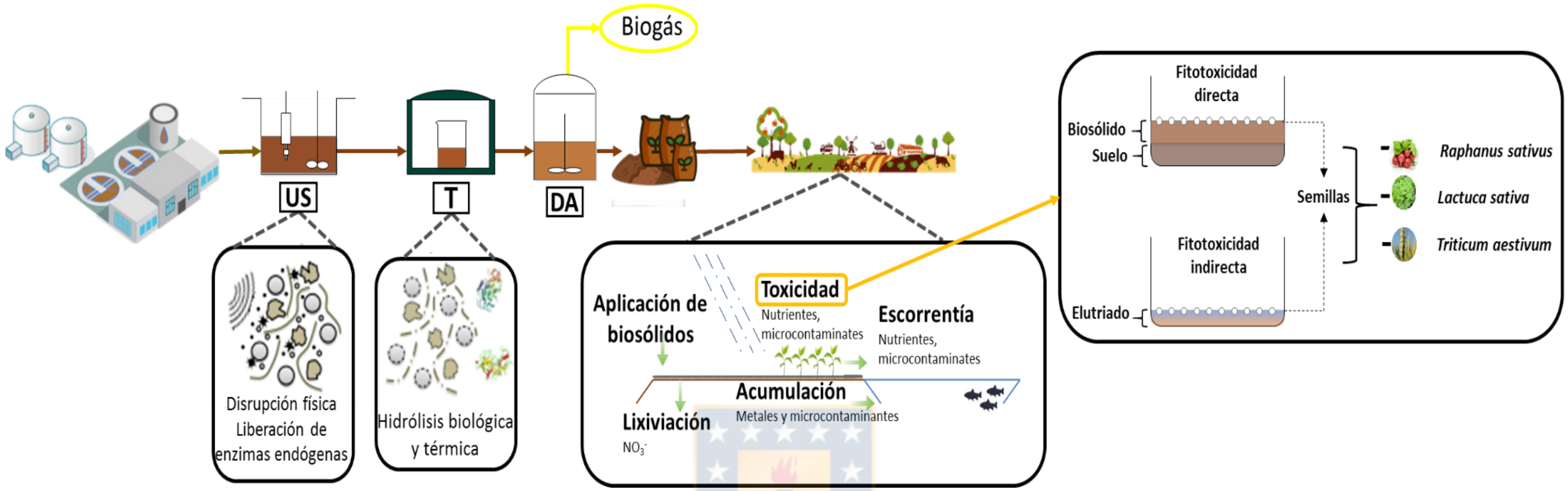

Figura 1. Diagrama del proceso de digestión anaerobia avanzada evaluado en esta investigación. US: pre-tratamiento de ultrasonido; T: pre-tratamiento térmico; DA: digestión a anaerobia.

CAPÍTULO II

MARCO TEÓRICO

1. Tratamiento de aguas servidas y generación de lodos sanitarios

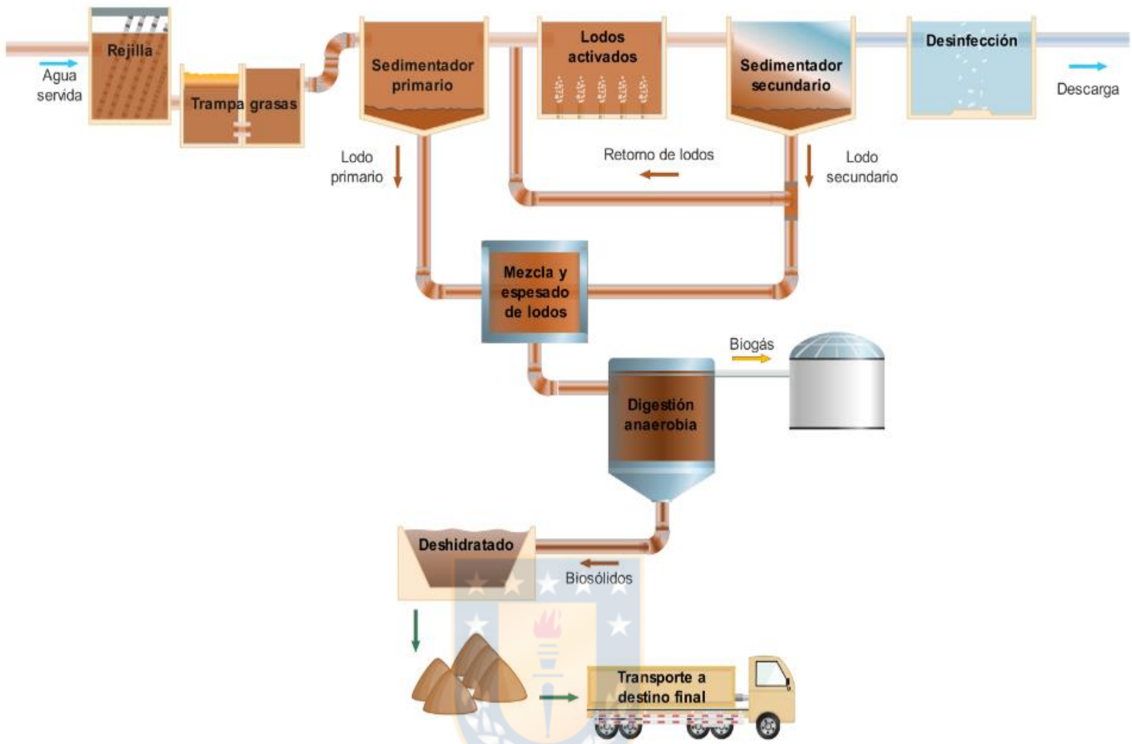

Figura 1. Diagrama del proceso de tratamiento de aguas servidas y lodos sanitarios.

1.1. Generación y tratamiento de aguas servidas

El tratamiento de las aguas servidas (AS) es fundamental para garantizar la estabilidad de los ecosistemas, así como para mantener la salud pública. La composición de las estas dependerá del asentamiento que las origine, pero comúnmente su composición variará entre los parámetros y valores presentados en la Tabla 1.

Tabla 1. Caracterización fisicoquímica y microbiológica típicas de las aguas servidas.

Parámetro	Unidad	Tipo de agua servida		
		Concentrada	Media	Diluida
Sólidos totales	mg/L	390	720	1230
Sólidos suspendidos totales	mg/L	120	210	400
Sólidos suspendidos volátiles	mg/L	95	160	315
Sólidos disueltos totales	mg/L	270	500	860
Sólidos disueltos volátiles	mg/L	110	200	340
Sólidos sedimentables	mg/L	5	10	20
Demanda bioquímica de oxígeno	mg O_2/L	110	190	350
Carbono orgánico total	mg/L	80	140	260
Demanda química de oxígeno	mg O_2/L	250	430	800
Nitrógeno total	mg/L	20	40	70
Nitrógeno orgánico	mg/L	8	15	25
Nitrógeno amoniacal	mg/L	12	25	45
Fósforo total	mg/L	4	7	12
Fósforo orgánico	mg/L	1	2	4
Fósforo inorgánico	mg/L	3	5	10
Grasas y aceites	mg/L	50	90	100
Compuestos orgánicos volátiles	mg/L	< 100	100 - 400	> 400
Coliformes totales	NMP/100mL	10^6-10^8	10^7-10^9	10^7-10^{10}
Coliformes fecales	NMP/100mL	10^3-10^6	10^4-10^6	10^5-10^8
Ooquistes de *Crystoporidium*	NMP/100mL	10^{-1}-10^0	10^{-1}-10^1	10^{-1}-10^2
Quistes de *Giarda lambia*	NMP/100mL	10^{-1}-10^1	10^{-1}-10^2	10^{-1}-10^3

NMP: número más probable. Fuente: Tchobanoglous et al. (2003)

El tratamiento del AS se da mediante una serie de etapas, dónde cada una de estas puede consistir en uno o más procesos de tratamiento (Spellman, 2009). La cantidad de etapas de tratamiento requeridas dependen en gran medida de la calidad necesaria de las aguas tratadas para poder ser vertida al cuerpo de agua receptor. Las principales etapas en el tratamiento de las aguas servidas son: tratamiento preliminar, tratamiento primario, tratamiento secundario, tratamiento terciario o avanzado, desinfección y tratamiento de lodos sanitarios (Figura 1); las cuales se describen brevemente en la Tabla 2.

Tabla 2. Principales etapas del tratamiento de aguas servidas.

Etapa	Objetivo/características	Tecnología usada
Preliminar o pre-tratamiento	Remoción de materiales de gran tamaño o que puedan afectar equipos o etapas siguientes, tales como: ramas, arenas, grasas y aceites.	Rejillas, cribado, tornillo sin fin, flotación
Primario	Remoción de sólido sedimentables. Reducción de la concentración de sólidos suspendidos entre un 40-70% y entre un 30-40% de la DBO. El material sedimentado se conoce como lodo sanitario primario.	Sedimentador o clarificador
Secundario	Remoción de la materia orgánica disuelta y coloidal por medio de la acción microbiológica del reactor biológico, entre un 85-95% de la DBO. Posterior al reactor biológico se encuentra un sedimentador donde se obtiene una corriente de agua depurada y otra de lodos (lodos sanitarios secundario). Una fracción de lodos secundarios es recirculada al reactor biológico y la otra es purgada.	Lodos activados, lagunas facultativas, biodiscos, digestor anaerobio
Terciario	Remoción de metales, compuestos químicos específicos o nutrientes como nitrógeno y fósforo.	Coagulación-floculación
Desinfección	Remoción de microorganismos para eliminar o reducir la posibilidad de enfermedades cuando las aguas tratadas sean descargadas.	Aplicación de cloro, ozono o luz ultravioleta
Lodos sanitarios	Estabilización de los lodos removidos del tratamiento primario y secundario.	Digestión anaerobia, compostaje, aplicación de cal

DBO: demanda biológica de oxígeno. Fuente: Gray and EBSCOhost (2010) ; Spellman (2009)

Durante el proceso de saneamiento de las aguas servidas se producen dos líneas de flujo, la primera corresponde a la línea de agua que considera desde el influente (agua servida) hasta el efluente (agua depurada) que es descargado al cuerpo de agua receptor. La otra corriente corresponde a la línea de lodos sanitarios, corriente semi-sólida producto de la mezcla de los lodos sanitarios (LS) primarios y secundarios (Figura 1). Para la línea de agua el sistema de lodos activados es una de las tecnologías ampliamente utilizada, por ejemplo, en Chile más del 60% de las PTAS emplean esta tecnología (Figura 2). En este tipo de PTAS alrededor del 60% de la materia orgánica presente en el agua servida será transferida a los LS (Tchobanoglous et al., 2003), los sistemas convencionales de lodos activados y los sistemas con aireación extendida tienen una producción de lodos secundarios sin espesar se estimada en 8.14 kg/hab-año y 6.15 kg/hab-año, respectivamente (Vera et al., 2013).

El manejo de los LS implica un costo entre 30-65% del costo de operación de la PTAS (Pérez-Elvira et al., 2006). La disposición de los lodos sanitarios sin estabilización en rellenos sanitarios disminuye la vida útil del predio y podría generar problemas de emisión de gases de efecto invernadero y lixiviados (Du, 2015). Entre las tecnologías de tratamiento de lodos sanitarios se incluyen secado térmico, aplicación de cal, compostaje, incineración y digestión anaerobia.

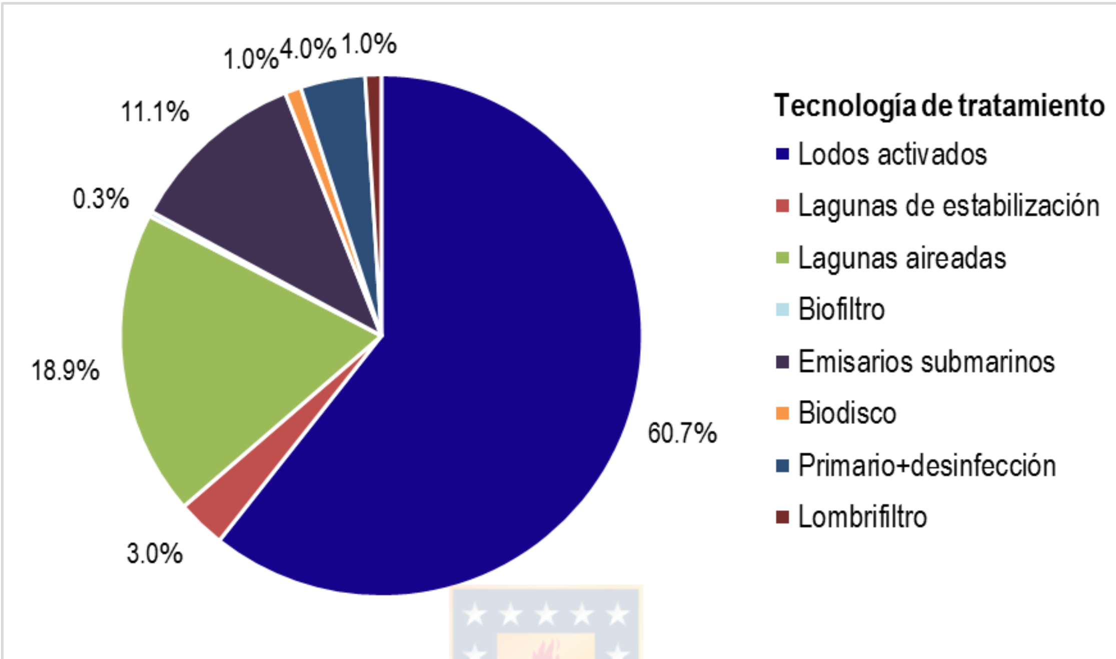

Figura 2. Tecnologías de tratamiento de aguas servidas en Chile. Modificado de SISS (2018).

1.2. Manejo de lodos sanitarios y de biosólidos

Los LS no se pueden disponer en el ambiente debido a los impactos ambientales asociados a su composición, tales como contaminación por metales, lixiviación de nutrientes, propagación de patógenos y contaminación por microcontaminantes orgánicos (Coors et al., 2016; Nogueirol et al., 2013; Petrie et al., 2014). Pese a ello, los lodos sanitarios tienen el potencial de ser valorizados si son estabilizados de forma adecuada, ya que la presencia de micro y macro-nutrientes y de materia orgánica estabilizada los convierte en una alternativa de fertilizante y enmienda de suelos (Goss et al., 2013; Hospido et al., 2010).

En el año 2017 Chile contaba con 265 PTAS las cuales generaron 653 405 m^3 de biosólidos, de los cuales el 49% fue generado en la Región Metropolitana y 12% en la Región del Biobío (SISS, 2018). En la Tabla 3 se presenta la generación regional de biosólidos para el año 2017, así como el destino de los biosólidos. Solo un 39% de los biosólidos generados son utilizados en la aplicación benéfica (uso agrícola o forestal), principalmente en la zona centro/sur de Chile.

Tabla 3. Destino final de los de biosólidos y estado de aplicación del reglamento de aplicación de lodos en Chile por región en el 2017.

Región	Generación (m^3/año)	Relleno sanitario / monorrelleno	Aplicación en suelo		PTAS con o sin autorización del reglamento D.S. 04/09*	
			m^3/año	%	Con	Sin
Arica y Parinacota	-	-	-	-	-	-
Tarapacá	7 304	7 304	-	0	5	-
Antofagasta	13 222	13 222	-	0	2	5
Atacama	24 759	24 759	-	0	2	5
Coquimbo	2 768	2 768	-	0	18	2
Valparaíso	43 678	43 678	-	0	5	20
Metropolitana	319 176	206 021	113 155	35	26	7
O'Higgings	43 390	17 706	25 684	59	11	13
Maule	42 345	38 644	3 701	9	27	4
Biobío	78 556	21 572	56 984	73	37	4
Araucanía	22 925	801	22 124	97	31	3
Los Ríos	15 282	6 766	8 516	56	10	1
Los Lagos	27 792	12 290	15 502	56	17	1
Aysén	6 342	296	6 046	95	8	-
Magallanes	5 867	0	5 867	100	1	-
Total	653 405	395 825	257 579	39	200	65

*D.S.04/09: Decreto Supremo 04/09 Reglamento para el manejo de lodos generados en plantas de tratamiento de aguas servidas.
PTAS: planta de tratamiento de agua servida. Fuente: SISS (2018)

2. Composición de los lodos sanitarios y biosólidos

Los lodo sanitarios (LS) son una mezcla compleja de microorganismos, materia orgánica sin digerir (papel, residuos de plantas, aceites y materia fecal), materia inorgánica y agua (Samolada and Zabaniotou, 2014). El material sin digerir contiene una mezcla compleja de moléculas provenientes de proteínas, péptidos, lípidos, polisacáridos, macromoléculas de plantas (ligninas o taninos) o estructuras alifáticas y microcontaminantes o contaminantes emergentes (la preocupación por estos es reciente y usualmente se encuentran en concentraciones $<10^{-6}$ g/L), como antibióticos, antidepresivos, pesticidas, detergentes, fertilizantes, preservantes, fragancias, hormonas, nanopartículas, retardantes de llama entre otros (Carballa et al. 2007; Artola-Garicano et al. 2003; Samolada & Zabaniotou 2014). La composición de los LS y de los biosólidos (BS) variará dependiendo de las características del agua servida y del tipo de tecnologías de tratamiento utilizada. A continuación, se presentan los valores reportados para los principales componentes de los LS y BS, materia orgánica, nutrientes, metales y microcontaminantes.

Tabla 4. Características fisicoquímicas de los lodos sanitarios y de biosólidos generados por diferentes tecnologías de estabilización.

Parámetro	Unidades	Biosólido					LS
		DAC	**DAA**	**Compost**	**Digestión aerobia**	**Otro**	
pH	-	5.8-8.4	5.4-6.8	5.8-8.1	6.1-9.5	5.6-10.4	5.5-7.8
Humedad	%	15.4-79.76	-	-	80.00-97.17	33.52-87.00	-
CE	mS/cm	0.93-9.91	-	4.3-9.31	1.5-8.9	1.2-5.84	0.005-5.19
MO	%	24-72	18.8-50.20	34.4-87.2	64.00-70.31	10-92	-
TOC	%	18.3-49.8	11.0-29.2	23.1	15.72-41.60	9.82-43.7	-
Nt	%	2.4-4.7	1.6-4.1	1.98-3.26	2.21-18.3	1.5-7.5	-
$N\text{-}NH_4$	g/kg	0.59-15.1	-	0.35-15.4	1.3-14.0	0.17-20.18	4.905-20.179
Pt	g/kg	13.6-60.0	9-53	12-39.2	7-34	4.2-101.8	10.2-60.8
K	mg/kg	2300-8000	-	-	1.9-9	1.17-6430	-
DQO	g/L	22	-	-	31.6	-	30-110

CE: conductividad eléctrica; Nt: nitrógeno total; NTK: Nitrógeno total Kjeldahl; Pt; fósforo total; DQO: demanda química de oxígeno; DAC: digestión anaerobia convencional; DAA: digestión anaerobia avanzada; LS: lodo sanitario. Fuente: (Adamcová et al., 2016; Alvarenga et al., 2016, 2008, 2007; Andrés et al., 2011; Artuso et al., 2011; Bonomo et al., 2016; Carballa et al., 2009; Carbonell et al., 2009; Carmen Antolín et al., 2010; Christofoletti et al., 2013, 2012; Domene et al., 2011, 2010; Fuentes et al., 2006, 2004; Groth et al., 2016; Hernández et al., 2016; Huguier et al., 2015; Lakhdar et al., 2012; Li et al., 2014; Lloret et al., 2016; Malara and Oleszczuk, 2013; Mattana et al., 2014; Mohamed et al., 2016; Murakami et al., 2009; Nafez et al., 2015; Oleszczuk, 2010, 2008a; Oleszczuk et al., 2012a, 2012b; Oleszczuk and Hollert, 2011; Renaud et al., 2017; Roig et al., 2012; Rossini-Oliva et al., 2017; Samolada and Zabaniotou, 2014; Singh and Agrawal, 2008)

Es común para lodos primarios que los sólidos totales (ST) fluctúen entren 2-8%, las grasas entre 13-65% de ST, las proteínas entre 20-30% de ST, el nitrógeno entre 1,5-4,0% de ST y el fósforo entre 0,2-0,6% de ST (Speece, 2008). En el caso del lodo secundario, los ST fluctúan entren 0,8-1,2%, las grasas entre 5-12% de ST, las proteínas entre 32-41% de ST, el nitrógeno entre 2,4-5,0% de ST y el fósforo entre 0,6-2,3% de ST (Speece, 2008). En la Tabla 4 se presentan otras características fisicoquímicas relacionadas con el contenido de materia orgánica (estimada por medio de la DQO) y contenido de nutrientes (nitrógeno y fósforo) de los LS y BS reportada en la literatura.

Los metales pueden estar solubles, adsorbido en la materia orgánica o inorgánica, como enlace orgánico o inorgánico, o como residuo (Du, 2015). En la Tabla 5 se presentan una recopilación de las concentraciones de metales y no metales en los LS y BS reportadas en literatura. Ramírez et al. (2008) muestran que la concentración de metales entre los LS deshidratados, BS estabilizado mediante compostaje y BS estabilizado mediante secado térmico, presentan contenidos similares para Pb (78-92 mg/kg), Zn (890-1028 mg/kg), Cd (3.1-3.5 mg/kg), Hg (2.13-2.51 mg/kg), Ni (45-76 mg/kg) y Cu (798-933 mg/kg), excepto para el Cr (54-127 mg/kg), el cual tuvo una concentración mayor (aproximadamente un 42%) en el BS estabilizado por secado térmicamente. Por otra parte, Carballa et al. (2009) evaluaron el impacto de la DAA (pre-tratamiento alcalino, térmico y ozono), donde los contenidos de metales se mantuvieron dentro del mismo rango en los BS y en los LS: Cu (170-680 mg/kg), Ni (32-152 mg/kg), Cr (19-318 mg/kg), Fe (4370-25370 mg/kg), Zn (360-2400 mg/kg), Pb (66-167 mg/kg), Cd (<1-3 mg/kg) y Hg (1.0-2.2 mg/kg).

Los LS y los BS contienen organismos patógenos como virus (entéricos y bacteriófagos), bacterias (salmonela, clostridium, estreptococos fecales, coliformes fecales y totales), protozoos (*Giardia lamblia*, *Ascaris*) y helmintos (*Trichuris pss*, *Toxocara*) (EPA, 2003). En los LS se han reportado concentraciones entre 10^5-10^7 NMP (número más probable) por gramo de sólidos totales (ST) de *T. coliforms*, 10^2-10^6 NMP/g ST de *E .coli*, 10^2-10^7 NMP/g ST de *F. streptococcus*, 10^4-10^6 NMP/g ST de *C. perfringens* y la presencia de *Salmonella spp* (Carballa et al., 2009; Corrêa et al., 2016).

Tabla 5. Concentración de metales y no metales en los lodos sanitarios y biosólidos (mg/kg)

Parámetro	Lodo sanitario	Biosólidos
Cu	174-331	76-2013
Ni	13-64	15-228
Cr	12-244	19-981
Fe	2080-24380	4370-30200
Zn	342-1035	360-4140
Pb	1-124	24-450
Cd	1-4	1-23
Hg	1-2	1-3
Mn	220	160-2621
Ba	518	201-454
As	1	7-17
Ca	114-8221	2322-41190
K	10-4322	2-10122
Mg	3761	994-10248
Na	12-2764	445-1114
Mo	<1	7
S	18306	1200-13855
Se	<1	3-50

Fuente: Adamcová et al. (2016); Arata et al. (2015); Carballa et al. (2009); Celis et al. (2008); Corrêa et al. (2016); Lesty (2003); Li et al. (2014); Nafez et al. (2015); Ramírez et al. (2008); Ros et al. (2015); Samolada & Zabaniotou (2014); Singh & Agrawal (2008); Zoghlami et al. (2016)

Entre los padecimientos causados por bacterias presentes en LS están: salmonelosis (*Salmonella sp.*), disentería (*Shigella sp.*) y gastroenteritis (*Escherichia coli*, *Campylobacter jejuni, Yersinia sp.*); entre los causados por virus: infecciones hepáticas (Hepatitis A), gastroenteritis (Norwalk, Rotavirus, reovirus, astrovirus, caliciviruses), diarrea (Norwalk, Rotavirus, Echovirus) y poliomelitis (poliovirus); entre las causadas por protozoarios: gastroenteritis (*Cryptosporidum*), giardiasis (*Giardia lambia*), diarrea (*Balantidium coli*) y toxoplasmosis (*Toxoplasma gondii*); y los causados por helmintos (*Ascaris lumbricoide, Ascaris suum, Trichuris trichiura, Toxocara canis, Taenia sanginafa, Taenia solium, Necator americanus, Hymenolepis nana*): dolor abdominal, vómito, cansancio, nerviosismo y anorexia (EPA, 2003).

Los BS deberían contener una menor cantidad de patógenos respecto al LS parental. La disminución de patógenos dependerá del tipo de tecnología utilizada en la estabilización de los LS y de la salud de la población. Por ejemplo, se presentan reducciones entre 0.5-4.0 log en bacterias para BS estabilizados por medio de DA, digestión aerobia, secado con aire y aplicación con cal, mientras que se podrían lograr reducciones entre 2-4 log por medio de compostaje (EPA, 2003). En el caso de virus, reducciones entre 0.5-2.0 log son posibles con DA o digestión aerobia y entre 2-4 log con compostaje o uso de cal (EPA, 2003). En general, en BS se reportan contenidos de *C. perfringens* entre 10^{2}-10^{5} NMP/g ST, coliformes fecales entre 10^{0}-10^{4} NMP/g ST, *T. coliforms* ente 10^{-2}-10^{8} NMP/g ST, *E. coli* entre 10^{-2}-10^{4} NMP/g ST, *F. streptococcus* 10^{-1}-10^{5} NMP/g ST y entre 0-13 huevos viables de helmintos por 10g ST (Arata et al., 2015; Carballa et al., 2009; Corrêa et al., 2016; Lesty, 2003; Nafez et al., 2015).

Carballa et al. (2009) evaluaron el impacto de la DAA (pre-tratamiento alcalino, térmico y ozono) reportando mejoras en la eliminación (>90%) de *T. coliforms*, *E. coli*, *F. strptococcus* y *Salmonella spp*., tanto en procesos con DAC y con DAA, excepto para la eliminación de *C. perfringens* (>70% en DAA y entre 28-56% en DA convencional). En general, en el caso del uso de DAA, se han reportado disminuciones o remociones de *Streptococcus fecalis* (pre-tratamiento térmico <100°C y adición alcalina), de coliformes fecales y *E. coli* (ultrasonido, microondas, ozono, adición alcalina y adición ácida), de *C. perfringens* (microondas y adición alcalina) y *Salmonella spp* (microondas, adición alcalina y adición ácida) (Neumann et al., 2016).

El uso extendido de compuestos químicos ha provocado que estos sean omnipresentes en las aguas servidas a nivel mundial y por ende entren en las plantas de tratamiento de aguas servidas (PTAS) y se estén presentes en los LS y BS (Bergersen et al., 2012; Malmborg and Magnér, 2015; Stasinakis, 2012). El destino de los microcontaminantes en las PTAS depende de varios factores como las características físico-químicas del compuesto (peso molecular, hidrofobicidad, solubilidad en agua, pk_a, biodegradación y biodisponibilidad), características de las aguas servidas y de los LS generados (pH, contenido de materia orgánica y cationes), parámetros operacionales de la PTAS (presencia o ausencia de sedimentador primario, tiempo de retención hidráulico y de lodos, tipo de tratamiento secundario, carga orgánica) y la frecuencia de uso de los compuestos por parte de la población (Carballa et al., 2006; Samaras et al., 2014; Verlicchi & Zambello, 2015).

Por ejemplo, el ibuprofeno, metoprolol y trimetoprima se particionan en la fase acuosa, la carbamazepina y diclofenaco pueden encontrarse entre las fases acuosa y sólida (Carballa et al., 2007; Malmborg & Magnér, 2015). Mientras que compuestos estrogenados, paroxetina, citalopram, fluoxetina, sertralina, triclosán, triclorocarban, bisfenol A, norfloxacín, ofloxacín y criprofloxacín son afines a la fracción sólida (Bergersen et al., 2012; Malmborg & Magnér, 2015; Petrie et al., 2014). En general, las concentraciones de microcontaminantes en los LS y BS reportadas en la literatura se presentan en la Tabla 6.

Tabla 6. Concentraciones de microcontaminantes en lodos sanitarios y biosólidos (µg/kg).

Microcontaminante		**Lodo Sanitario**	**Biosólido**
Claritromicina, eritromicina, ketoconazol, ofloxacin, oxitetraciclina, sulfamethoxazole, triclosan, triclorocarban, tetraciclina, trimetroprima, ciprofloxacín, norfloxacín	antimicrobiótico	8-80000	<1-133000
Citalopram, fluoxetina, sertralina	antidepresivo	19-270	39-770
Carbamazepina	antiepiléptico	300	<1-1891
Diclorofenaco, ibuprofeno, naproxeno	antiinflamatorio	10-1185	<1-1738
Tonalida, galaxolide	fragancia	10200-187000	16000-81000
Estrona, 17β-estradiol, estradiol, 17α-etinilestradiol, progesterona, testosterona	hormona	<1-887	2-22000
Sulfonatos de alquilbenceno lineales, nonilfenoles	surfactante	4900-2200000	25000-5572000

Fuente: Al-Rajab et al. (2015); Carballa et al. (2007); Carballa et al. (2009); Combalbert & Hernandez-Raquet (2010); Malmborg & Magnér (2015); McNamara et al. (2012); McNamara et al. (2014); Ramírez et al. (2008); Rastetter & Gerhardt (2015); Ros et al. (2015); Shargil et al. (2015); Symsaris et al. (2015); Verlicchi & Zambello (2015); Wu et al. (2015); Zhang et al. (2015)

Entre los compuestos que han reportado una reducción durante la DAC están: paroxetina (98%), naproxeno (80%), sulfamethoxazole (80%) y roxitromicina (80%) (Bergersen et al., 2012; Carballa et al., 2006; McAvoy et al., 2015). Otros como fluoxetina (reducción 32%), fluvoxamina (53%), sertralina (38%), diazepam (50%) e ibuprofeno (40%) podrían tener un mayor potencial de acumulación (Bergersen et al., 2012; Carballa et al., 2006; Malmborg & Magnér, 2015). Sin embargo, existen contradicciones entre investigaciones, por ejemplo, en la degradación de compuestos estrogénicos algunos estudios reportan remoción en la digestión anaerobia mesofílica o termófila con o sin pre-tratamiento (Carballa, et al., 2007; Carballa et al., 2006). Otros estudios reportan remoción solo con el uso de pre-tratamiento (Malmborg & Magnér, 2015; Muz et al., 2013). Otros estudios concluyen que la remoción de este tipo de compuestos no se logra en ninguna condición de DAC o DAA (Chawla et al., 2014; Holbrook et al., 2002).

3. Digestión anaerobia convencional y avanzada de lodos sanitarios

La digestión anaerobia es una alternativa interesante para la estabilización porque permite una reducción del volumen de los LS, tiene requerimientos de energía relativamente bajos, produce biogás que puede ser aprovechado, y genera un lodo sanitario estabilizado (biosólido, BS) que podría tener un uso benéfico (McNamara et al., 2012; Stasinakis, 2012; Tiwary et al., 2015).

3.1. Descripción del proceso de digestión anaerobia convencional y avanzada

La digestión anaerobia es un proceso que sucede en condiciones estrictas de ausencia de oxígeno, donde la materia orgánica compleja es degradada por un consorcio microbiano formando un digestado y biogás, este último compuesto principalmente por metano y dióxido de carbono (Speece, 2008). El ecosistema anaeróbico es el resultado de una compleja interacción de varios microorganismos de diferentes especies, cada cual con aspectos fisiológicos, nutricionales, cinéticas de crecimiento y sensibilidad a factores ambientales propias (Appels et al., 2008). A partir de análisis de metagenómica de lodos anaerobios se reporta que entre el 83-95% de las secuencias genómicas corresponden al dominio *Bacteria*, entre 2-5% a *Archaea* y entre el 0.3-2% al *Eukaryota* (Ying Yang et al., 2014). Encontrando en el dominio *Bacteria* a las *Proteobacteria, Bacteroidetes* y *Firmicutes* como los filos más abundantes (Traversi et al., 2012; Ying Yang et al., 2014). Los organismos metanogénicos más abundantes se encuentran los géneros *Methanosaeta* y *Methanosarcina* (Ying Yang et al., 2014). Durante la digestión anaerobia se distinguen 4 etapas principales (Figura 3), las cuales serán brevemente descritas a continuación (Appels et al., 2008; Speece, 2008; van Lier et al., 2008):

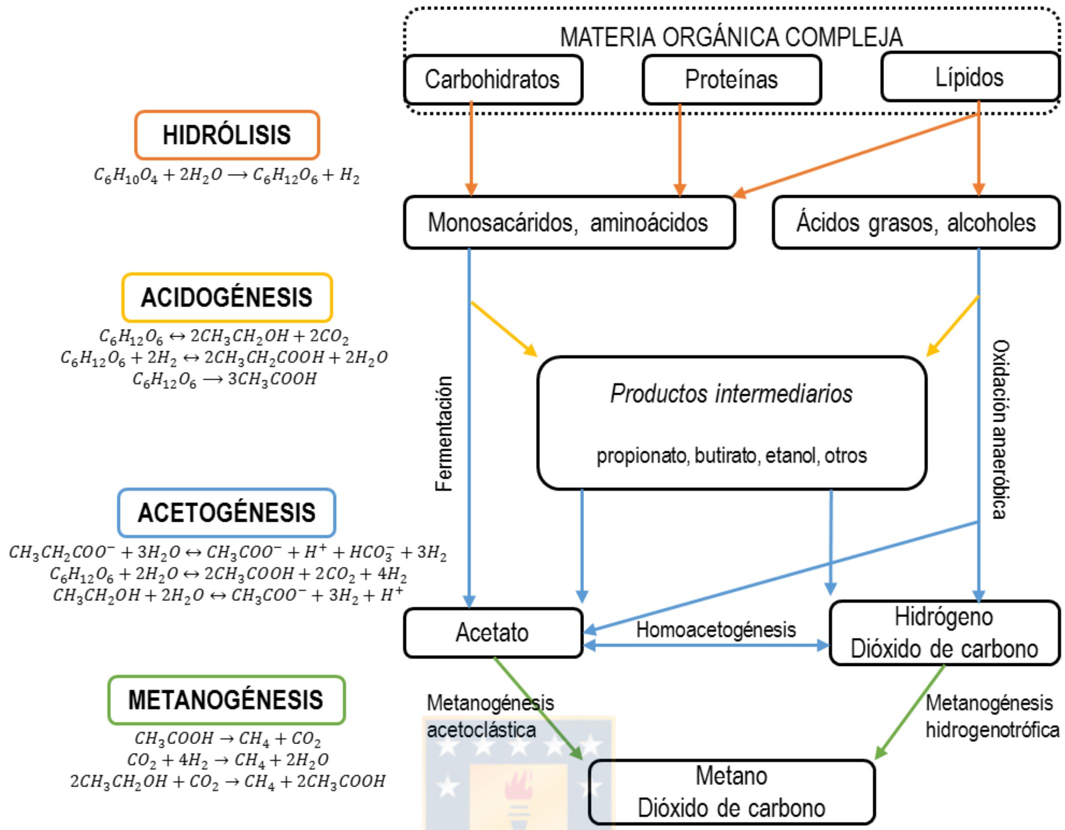

Figura 3. Representación esquemática del proceso de digestión anaerobia y los microorganismos involucrados. Modificado de Speece (2008).

Hidrólisis: es un proceso enzimático en el que se da la conversión de carbohidratos, proteínas y lípidos en monosacáridos, aminoácidos y ácidos grasos de cadena larga, respectivamente. La transformación se da por exoenzimas, la degradación de los carbohidratos es producto de la acción de las celulasas que los degradan en monosacáridos, disacáridos y trisacáridos; la degradación de proteínas es mediada por proteasas y peptidasas que las transforman en aminoácidos; la catabolización de lípidos es mediada por lipasas que los transforman en ácidos grasos de cadenas largas y glicerol (Figura 4).

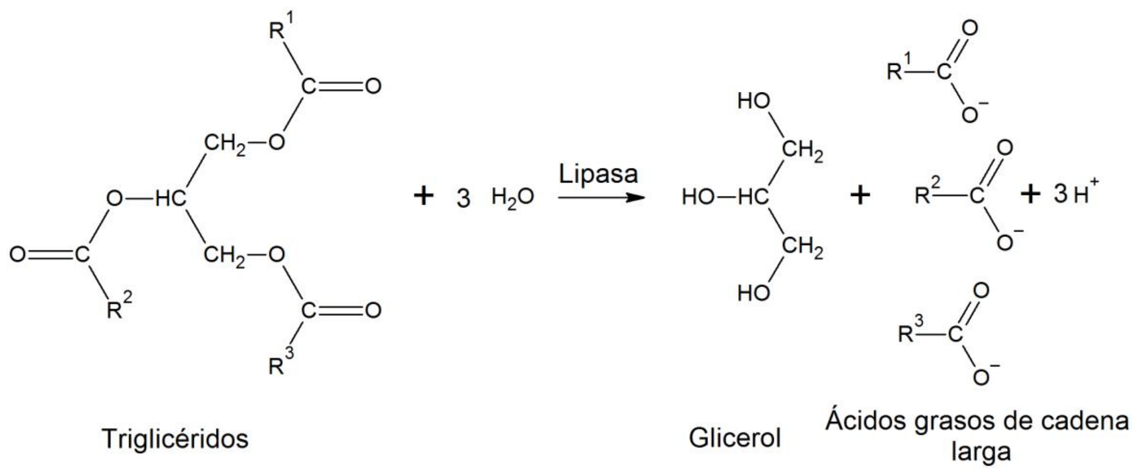

Figura 4. Representación esquemática de la hidrólisis de lípidos en ácidos grasos de cadena larga. Modificado de van Lier et al. (2008).

Acidogénesis: los monosacáridos, aminoácidos y ácidos grasos de cadena larga ingresan por la membrana celular de las bacterias y son oxidados anaeróbicamente o fermentados en una mezcla de ácidos grasos volátiles, alcoholes, ácido láctico, hidrógeno y dióxido de carbono. En esta etapa actúan tanto microorganismos hidrolíticos como no-hidrolíticos. En el proceso de digestión anaerobia la etapa acidogénica es la de mayor taza de conversión de sustratos con una energía de libre de Gibbs ($\Delta G°'$) más favorable, resultando en razones de crecimiento bacteriano entre 10 y 20 veces mayor que los organismos metanogénicos (Tabla 7).

Tabla 7. Propiedades cinéticas promedio de los organismos acidogénicos y metanogénicos.

Etapa	Tasa de conversión (g DQO/ g SSV d)	Y (g SSV/ g DQO)	K_s (mg DQO/L)	μ_m (1/d)
Acidogénesis	13	0.15	200	2.00
Metanogénesis	3	0.03	30	0.12
General	2	0.03-0.18	-	0.12

DQO: demanda química de oxígeno; SSV: sólidos suspendidos volátiles; Y: tasa de crecimiento bacteriano, Ks: constante de afinidad por el sustrato; μm: tasa de crecimiento específico. Fuente: van Lier et al. (2008)

Acetogénesis: los ácidos grasos de cadena corta son convertidos en acetato, hidrógeno y dióxido de carbono. Los intermediarios principales en la acetogénesis son el propionato y el butirato, pero también lactato, etanol, metanol, hidrógeno y dióxido de carbono, también son convertidos en acetato. En esta etapa es de vital importancia la relación sintrófica de las bacterias acetogénicas generadoras de hidrógeno y de las bacterias metanogénicas hidrogenotróficas que lo consumen, esto debido a que alrededor del 70% del metano se produce vía organismos metanogénicos acetoclásticos y la oxidación del ácido propiónico y del butirato requieren presiones parciales de hidrógeno bajo 10^{-4} y 10^{-3} atm, respectivamente.

Metanogénesis: en la última etapa de la digestión anaerobia el hidrógeno y el acetato son convertidos en metano y dióxido de carbono por arqueas metanogénicas. Los organismos metanogénicos son anaerobios estrictos con un espectro muy corto de sustratos: acetato, metilaminas, metanol, formiato, hidrógeno, dióxido de carbono o monóxido de carbono. El hidrógeno es convertido por microorganismos metanogénicos hidrogenotrópicos y el acetato por metanogénicos acetoclásticos. Los primeros poseen tasas de crecimiento entre 4 a 12 veces mayores que los segundos (Tabla 8). Sin embargo, la mayor cantidad de metano es generado por vía acetoclástica.

Tabla 8. Reacciones metanogénicas más importantes, su correspondiente energía libre de Gibbs ($\Delta G°'$) y algunas propiedades cinéticas.

Etapa	Reacción	$\Delta G°'$ (kJ/mol)	$\mu_{máx}$ (1/d)	T_d (d)	K_s (mg DQO/L)
Metanogénesis acetotrófica	$CH_3^- COO^- + H_2O \longrightarrow CH_4 + HCO_3^-$	-31	0.12* 0.71**	5.8* 1.0**	30* 300**
Metanogénesis hidrogenotrópica	$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$	-131	2.85	0.2	0.06

* *Methanosarcina sp.*; ** *Methanosaeta sp.*; DQO: demanda química de oxígeno; Td: tiempo de duplicación, Ks: constante de afinidad por el sustrato; μmáx: tasa de crecimiento específico máximo. Modificado de van Lier et al. (2008).

En la digestión anaerobia de LS, la hidrólisis es la etapa limitante debido a la presencia de materia orgánica particulada, compuestos de alto peso molecular y sustancias poliméricas extracelulares (Dohányos et al., 2004; Gianico et al., 2013). Para superar esta limitación en la digestión anaerobia convencional (DAC) de LS se han incorporado etapas previas, pre-tratamientos, dando lugar a lo que se conoce como digestión anaerobia avanzada (DAA). Los pre-tratamientos se clasifican en procesos físicos, químicos, biológicos o combinados, los cuales pueden mejorar la tasa de digestión y de biodegradabilidad de los LS (Neumann et al., 2016).

3.2. Pre-tratamiento de lodos sanitarios

Desde finales de los 1970's se ha estudiado la aplicación de pre-tratamientos térmicos para mejorar la biodegradabilidad de los lodos sanitarios previo a la DA (Haug et al., 1978). Actualmente la DAA ha recibido considerable atención y se han incorporado una amplia gama de pre-tratamientos los cuales se encuentran en diferentes etapas de desarrollo y aplicación (Ariunbaatar et al., 2014; Carlsson et al., 2012; Carrère et al., 2010; Neumann et al., 2016). A continuación, se describen los principales tipos de pre-tratamiento físicos, químicos y biológicos y en la Tabla 9 se muestra un resumen de las condiciones operacionales y principales efectos de los tipos de pre-tratamiento.

Pre-tratamiento físico: en esta categoría están aquellos basados en fenómenos hidrodinámicos (térmico), hidromecánicos (ultrasonido, cambios de presión, molienda) y electromagnéticos (pulsos eléctricos, microondas). El pre-tratamiento hidromecánico involucra el uso de la fuerza para romper las células de los microorganismos, aumentando la superficie de contacto y mejorando el contacto entre el sustrato y las bacterias anaerobias (Ariunbaatar et al., 2014), para este pre-tratamiento se reportan aumentos en la producción de biogás entre 6-260% y reducciones de sólidos entre 6-106% (Neumann et al., 2016). El uso de sistemas de baja temperatura (70-100°C), alta temperatura (>100°C), y secuencias de congelación/descongelación corresponden a pre-tratamientos hidrodinámicos, en los que se reportan aumentos en la producción de biogás entre 10-984% y reducción de sólidos entre 30-615% (Neumann et al., 2016); estos sistemas utilizan el calor para destruir las paredes celulares del lodo sanitario y acelerar la DA (Ariunbaatar et al., 2014).

Pre-tratamiento químico: en esta categoría se agrupan los pre-tratamientos basados en la adición de compuestos ácidos (ácido periacético), alcalinos (hidróxido de sodio) u oxidantes (ozono, reactivo Fenton, peróxido de hidrógeno) que faciliten la hidrólisis del lodo sanitario (Appels et al., 2011; Carrère et al., 2010). Este tipo de pre-tratamiento logra aumentos en la producción de biogás entre 11-200% con reducciones de sólidos entre 12-72% (Neumann et al., 2016).

Pre-tratamiento biológico: el pre-tratamiento biológico incluye tanto métodos anaerobios como aerobios, así como la adicción de enzimas específicas como peptidasa, carbohidrolasa y lipasa, o bien el uso de la actividad enzimática endógena de los lodos sanitarios, particularmente de los lodos secundarios (Ariunbaatar et al., 2014), obteniendo aumentos en la producción de biogás entre 3-85% y reducción de sólidos entre 4-53% (Neumann et al., 2016).

Tabla 9. Resumen de los principales pre-tratamientos, condiciones operacionales y efectos sobre la digestión anaerobia.

Tipo	Pre-tratamiento	Condiciones del pre-tratamiento	Principales efectos en la DA: Aumento (%) Biogás	Principales efectos en la DA: Aumento (%) Metano	Principales efectos en la DA: Reducción SV (%)
Térmico	Alta temperatura (100-210°C)	100-210°C; 20-60 min; 3-21 bar	25-150	-	7-105
	Baja temperatura (<100°C)	70-95°C; 15 min-7 d	10-984	2-10	10-40
Físico	Congelado/descongelado	-80 a -10°C; 24 h congelado; 12-15 h descongelado	52	-	4
	Ultrasonido	112-108000 kJ/kg ST; 60-480 W; 20-200 kHz; 0.5-60 min	4-83	-	6-47
	HAP	50-600 bar; 300-3380 kJ/kg ST; 1-2 ciclos	17-115	-	7-138
	Microondas	336000 kJ/m^3; 30-175°C; 800-1250 MW; 2450 MHz	16-50	-	23-48
	Electrólisis y pulso eléctrico	1.1-34 kWh/m^3; 8-30kV/cm	-	10-100	9
	Irradiación dual	55°C; 100 µmol/m^{2}s; 8-48 h; 60 W	-	12-42	53-73
	Cavitación	12 bar; 15-90 min; ciclos de 3 min	94	-	-
Químico	Alcalino	8-12 pH; 30 min-8 d	-	13-120	>133
	Ácido	1-6 pH; 1-24 h	12-32	-	5
	Ozono	0.1-0.16 g O_3/ g ST; 1-3h	8-200	-	-
	Fenton	5-100 g H_2O_2/ kg; 0.4-5.0 g Fe(II); 60 min; pH 3 (H_2SO_4)	-	19-30	31-72
	POMS/DMO peroxidación	30-60 g POMS/kg; 330-660 g DMO/kg; 1 h	11-33		79-185
Biológico	Digestión aerobia	55-65°C; 0.5-2 d; 5 L_{aire}/min	-	10-79	11-67
	Adición de enzimas	0.06-18% de enzimas; 37-50°C; 4 h-11 d	12	-	1-16
	Digestión dual	37-70°C; 0.5-6 d	11-50	-	10-53
	Auto-hidrólisis	42-55°C; 12-48 h	-	16-23	-
Combinado	Alta temperatura-químico	2-12 pH; 115-170°C; 5-35 min	-	40-154	72
	Baja temperatura-químico	8-11 pH; 50-90°C; 0.5-33 h	-	20-70	-
	Microondas-alcalino	10-12.5 pH; 1-51 min; 100-210°C	44-228	17-228	28-262
	Alta presión-ozono	6-11 g O_3/ g ST; 5-20 ciclos; 690-1040 kPa; 16 min	47	107	16-41
	Ultrasonido-alcalino	8-13 pH; 6000-30000 kJ/kg ST; 20 kHz	38-55	-	-
	Ultrasonido-ozono	1000-12000 kJ/ kg ST; 0.012-0.12 g O_3/ g ST; 20 kHz	26-36	15-100	18-21
	Mecánico-alcalino	2500 rpm; 11-13 pH; 30-90 min	-	84-832	-
	Electroquímico	7-12 pH; 5-20 V; 40 min	63	20	17
	Microondas-térmico	2450 MHz; 96°C microondas; 55°C térmico; 5 d	94-100	-	99-106
	Alta presión-químico-térmico	10-11 pH; 6000-12000 PSI; 30 min; 55°C; 2 d	-	45-81	5-21
	Ultrasonido-térmico	24 kHz; 0.4-0.5 kWh/kg ST; 37°C; 3-5 d	-	8-33	40-70
	Físico-químico-térmico	9-11 pH; 12 bar; 30 min; 35°C, 5-9 d	13-28	-	56

DA: digestión anaerobia; SV: sólidos volátiles; HAP: homogenización a altas presiones ST: sólidos totales; POMS: peroximonosulfato; DMO: dimetildioxirano; BS: biosólido. Modificado de Neumann et al. (2016)

Pre-tratamiento combinado: Considerando un balance energético, no todos los pre-tratamientos a escala de laboratorio son energéticamente factibles en una escala real (Cano et al., 2015). Para superar esta barrera se propone el uso de sistemas combinados, estrategia que tiene el potencial de generar efectos sinérgicos en la biodegradabilidad y solubilización de los lodos sanitarios (Dhar et al., 2012; Neumann et al., 2018). Se ha investigado la aplicación de procesos termo-químicos (Harris and McCabe, 2015; Kim et al., 2003), físico-químicos (Tian et al., 2015; Tyagi and Lo, 2011) y físico-biológicos (Gianico et al., 2013; Neumann et al., 2017). Esta última combinación resulta interesante porque busca favorecer la actividad enzimática endógena del lodo sanitario sin la adicción de compuestos. Esto consecuencia a que en la etapa física se da la disrupción parcial de las sustancias poliméricas extracelulares, las cuales cuentan con una serie de enzimas como proteasas, amilasas, lipasas, fosfatasas, aminopeptidasas, dehidrogenasas y glucosidadas que podrían contribuir en la hidrólisis del lodo sanitario una vez que fuesen liberadas de la matriz de los flóculos y reactivadas durante una etapa subsecuente (Carvajal et al., 2013; Neumann et al., 2017).

En particular, la combinación ultrasonido y tratamiento térmico a bajas temperaturas (70-95°C) permite la disrupción física de los flóculos y brinda condiciones favorables para la reactivación de la actividad enzimática endógena (Carvajal et al., 2013; Neumann et al., 2017). Estudios previos reportan que esta secuencia de pre-tratamientos resultó en incrementos de 458 – 1030% y 252 – 674% en la concentración soluble de carbohidratos y proteínas, respectivamente (Neumann et al., 2017) y aumentos en la generación de biogás de 19% (Neumann et al., 2018).

4. Herramientas de evaluación de los biosólidos para su uso benéfico en suelo

El uso de BS presenta múltiples beneficios económicos y ambientales. Sin embargo, su implementación en la agricultura enfrenta un gran desafío: la seguridad alimentaria, ecosistémica y el riesgo asociado a los microcontaminantes (Wu et al., 2015). Es importante considerar que los análisis requeridos por las diferentes normativas no predice o considera efectos sinérgicos de las sustancias orgánicas e inorgánicas presentes en una muestra compleja como los BS (Corrêa et al., 2016; Kapanen & Itävaara, 2001), es decir, las consideraciones fisicoquímicas establecidas en las normativas no son suficientes para evaluar correctamente los BS. Por ejemplo, los análisis químicos son una metodología de control de contaminantes, pero no proveen información directa sobre los efectos biológicos del tóxico o de la mezcla de estos. Por otra parte, sistemas de monitoreo biológico permiten una evaluación ecotoxicológica pero no proveen la identidad del contaminante (Ramírez et al., 2008).

La toxicidad es el potencial relativo de una sustancia (tóxico) o combinación de ellas, de producir daño a los organismos vivos, es una cualidad multifactorial que depende de factores ambientales, ecológicos, presencia de otras sustancias y características fisicoquímicas del tóxico. Considerando la gran gama de microcontaminates que pueden estar presentes en los biosólidos (Ver Tabla 4 del Capítulo IV) y la multifactoriedad de la toxicidad, la evaluación de la carga de contaminantes es importante para la determinación de la calidad de los biosólidos.

La toxicidad se puede manifestar como toxicidad aguda (rápida y severa), subletal (debajo del nivel que causa la muerte directamente) o crónica (se manifiesta en un periodo de tiempo más tardío, días/años dependiendo del ciclo de vida del organismo). Los biosólidos aplicados en suelos pueden causar fitotoxicidad (toxicidad aguda), alteraciones en la biota del suelo (toxicidad aguda y/o crónica), translocación de contaminantes, o bien, pueden causar efectos toxicológicos a nivel molecular y celular como la disrupción endocrina.

La toxicidad de los contaminantes en biosólidos puede ser monitoreada por medio de herramientas de ecotoxicología y ecotoxicogenómica. Donde la ecotoxicogenómica se refiere a la integración de las ciencias basadas en la genómica y la ecotoxicología, por medio del uso de tecnologías basadas en arreglos de ADN, proteómicoa y metabolíomica (Oliveira et al., 2015). La ecotoxicología es la rama de la toxicología que estudia los efectos tóxicos causados por contaminantes en los diferentes niveles ecosistémicos (animales, plantas y microorganismos). En el caso de los biosólidos que van a ser aplicados en suelos, su efecto fitotóxico es un parámetro de toxicidad aguda (afecta las etapas iniciales de desarrollo vegetal, la germinación) de gran interés.

Los ensayos de fitotoxicidad son herramientas rápidas y de bajos requerimientos instrumentales para medir las respuestas biológicas (Oleszczuk and Hollert, 2011). Sin embargo, para evaluar correctamente la fitotoxicidad de los biosólidos se debe de tener en cuenta la influencia de la matriz seleccionada para los ensayos, ya que está influirá en el comportamiento de los contaminantes presentes (Ramírez et al., 2008). Si se utiliza la matriz completa, todo el biosólidos se denomina fitotoxicidad directa, por otra parte si se utiliza un extracto representativo se conoce como fitotoxicidad indirecta (Alvarenga et al., 2016; Kapanen et al., 2013).

La fitotoxicidad directa evita problemas durante las extracciones y es una representación más cercana a las condiciones reales de disposición de biosólidos, sin embargo el alto contenido de materia orgánica podría enmascarar algunos contaminantes (Alvarenga et al., 2016; Kapanen et al., 2013). Por ello, evaluar un extracto con agua o elutriado de los biosólidos podría aportar información toxicológica de interés, ya que esta fracción está asociada a los compuestos a con una mayor biodisponibilidad (Alvarenga et al., 2016). También es importante considerar que diferentes especies de plantas responden de manera diferente a un mismo grado de contaminación (Adamcová et al., 2016). Algunas de las plantas utilizadas para determinar la fitotoxicidad de los biosólidos son: *Brassica rapa L, Lolium perenne L, Trifolium pratense L, Allium cepa, Sinapis alba L, Lepidium sativum, Sorghum saccharatum, Avena sativa, Cucumis sativus, Hordeum vulgare L, Lactuca sativa* y *Trifolium pratense*.

5. Marco legal de la aplicación de biosólidos en el suelo

Para prevenir efectos adversos por la aplicación de BS en suelos en diversas partes del mundo se han desarrollado e implementado normas y guías de aplicación. Por ejemplo, en Australia, Europa, Estados Unidos y Chile se ha acogido la NSW-EPA (2000), CEC (1986), USEPA (1992) y DS04/00-MINSEGPRES (2009) respectivamente. En la Tabla 10 se muestran las concentraciones de metales consideras por las normativas mencionadas anteriormente.

Tabla 10. Regulaciones internacionales sobre el contenido de algunos metales (mg/kg) en los biosólidos para su disposición en suelo.

Metal (mg/kg)	Regulación NSW-EPA Australia	CEC Europa	USEPA Estados Unidos	DS04/00 Chile
Cd	3-32	20-40	85	8-40
Cu	100-2000	1000-1750	4300	1000-1200
Ni	60-300	300-400	420	80-420
Pb	150-500	750-1200	840	300-400
Zn	200-3500	2500-4000	7500	2000-2800

La normativa en Chile, el Decreto Supremo 04 específicamente, estable que un LS ha sido estabilizado si se ha reducido su potencial de atracción de vectores, cuyo principal parámetro es una reducción de al menos un 38% de sólidos volátiles (MINSEGPRES, 2009). El BS generado se puede clasificar en Clase A y Clase B. Los primeros son aptos para aplicación sin restricción; los segundos son aptos para aplicación benéfica, con restricciones de aplicación según tipo y localización de los suelos y cultivos. Para ser clasificado como Clase A el biosólido debe tener una densidad de coliformes menor a 1 000 NMP/g ST; o una densidad de *Salmonella sp*. menor a 3 NMP/4 g ST y un contenido de ova helmíntica viable menor a 1 /4g ST. Mientras los Clase B deben presentar una media geométrica del contenido de coliformes fecales menor que 2 000 000 NMP/g ST (MINSEGPRES, 2009).

Sin embargo, existe un vacío en los límites sobre la cantidad de microcotaminantes orgánicos en los BS para disponer en suelos (Petrie et al., 2014; Verlicchi and Zambello, 2015). Entre las pocas normas que consideran este tipo de compuestos está la Guía de utilización de compost y digestato de Suiza que contempla un valor límite para hidrocarburos aromáticos policíclicos (PAH: polycyclic aromatic hydrocarbons) de 4 mg/kg y 20 ng I-TEC/ mg/kg (I-TEC: International Toxicity Equivalents), por otra parte, el Ministerio de Ambiente Danés establece como concentración límite de PAH de 3 mg/kg, 1300 mg /kg de sulfonatos de alquilbenceno lineales y 10 mg/kg de nonilfenoles y en Austria los PAHs no pueden sobrepasar los 6 mg/kg y los bifenoles policíclicos los 0.2 mg/kg (Al Seadi and Lukehurtst, 2012). No obstante, se están realizando algunos esfuerzos para mejorar este aspecto, en Alemania por ejemplo, se planea implementar una directiva con valores límites para contaminantes orgánicos persistentes (POPs: persistent organic pollutants) (Rastetter and Gerhardt, 2015).

La toxicidad de los biosólidos, como la toxicidad de cualquier residuo orgánico, es el resultado de la combinación de múltiples factores. Investigar los efectos de los compuestos de los biosólidos en el ambiente, tanto en forma individual como una mezcla compleja, es trascendental para determinar la calidad de los biosólidos de manera integral, que permita tomar las medidas adecuadas para disponer y tratar este tipo de residuo.

CAPÍTULO III

HIPÓTESIS Y OBJETIVOS

1. Hipótesis

La digestión anaerobia avanzada de lodos sanitarios causa un cambio significativo favorable en la composición (características fisicoquímicas, microbiológicas y en la concentración de microcontaminantes) y en la fitotoxicidad del biosólido en comparación con el obtenido por digestión anaerobia convencional, propiciando preferencialmente el uso benéfico de dicho biosólido.

2. Objetivos

2.1 Objetivo general

Evaluar la influencia de la digestión anaerobia convencional y avanzada utilizando un pre-tratamiento secuencial de ultrasonido seguido de una etapa térmica a baja temperatura (55°C) en la composición (características fisicoquímicas, microbiológicas y en la concentración de microcontaminantes) y en la fitotoxicidad directa e indirecta de los biosólidos generados.

2.2 Objetivos específicos

- Determinar la influencia de la digestión anaerobia convencional y avanzada en el desempeño de la digestión y en las características fisicoquímicas y microbiológicas de los biosólidos generados.
- Evaluar la influencia de la digestión anaerobia convencional y avanzada en la concentración de microcontaminantes de los biosólidos generados.
- Evaluar la fitotoxicidad directa e indirecta de los biosólidos producto de un proceso de digestión anaerobia convencional y avanzada.

CAPÍTULO IV

ORGANIC MICROPOLLUTANTS IN SEWAGE SLUDGE: INFLUENCE OF THERMAL AND ULTRASOUND HYDROLYSIS PROCESSES PRIOR TO ANAEROBIC STABILIZATION

Reyes-Contreras C., Neumann P., Barriga F., **Venegas M.**, Domínguez C., Bayona J.M. and Vidal G. 2018. Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization. *Environmental Technology*. DOI: 10.1080/09593330.2018.1534892

Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization.

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Abstract

Organic micropollutants (OMP) in household and industrial wastewater are not efficiently removed by conventional treatment processes and a significant fraction ends in sludge. Proper valorization technologies become fundamental to attain sustainable sewage sludge management, with anaerobic digestion (AD) as one of the preferred strategies. However, it exhibits some limitations that can be overcome with pre-treatment processes. In this study, the influence of different pre-treatment configurations over OMP concentration and removal during AD was assessed. The incorporation of a sequential US – TT-PT resulted in decreased concentrations of 7 of the 9 detected compounds in biosolids compared to conventional AD digestate, with bisphenol-A and ter-octylphenol showing the opposite effect. The results suggest that the assessed PT could improve the removal of sequestered or highly hydrophobic compounds through their solubilization and increased bioavailability.

1. Introduction

Sewage treatment represents a fundamental mainstay for public health protection. Through successive physical, chemical and biological processes, efficient removal of solids, organic matter, nutrients and pathogens can be achieved. However, removal of organic micropollutants (OMP) that enter in the sewage system from household and industrial wastewaters is not always efficient, being influenced by the physicochemical characteristics of the pollutant and the treatment technology used (Luo et al., 2014). Furthermore, in most conventional treatment processes the fate of a significant fraction of the different OMP is the sludge generated during the depuration process (Kinney et al., 2006; Kupper et al., 2004; Martín et al., 2012).

Sewage sludge (SS) generated during activated sludge processes is characterized by high concentrations of solids (2 - 12% total solids for liquid sludge and 12 - 40% for dehydrated sludge), organic matter (55 - 85% of volatile solids in dry basis), pathogens (109 fecal coliforms/100 mL, 2500 - 70000 virus/ 100 mL, 200 – 1,000 Helminth/ 100 mL) and nutrients (> 8 mg P/ kg, > 30 mg N/ kg, > 3 mg K/ kg) (EPA, 1995; Maria et al., 2010). Therefore, proper treatment and disposal alternatives are necessary in order to avoid impacts such as pathogen spread, toxicological effects or uncontrolled input of nutrients to natural ecosystems. In this scenario, management of sludge can rise up to 50% of the total operational costs of current-technology depuration plants (Appels et al., 2008).

Therefore, proper valorization technologies become fundamental to attain sustainable SS management practices. One of the preferred strategies is the use of anaerobic digestion (AD) followed by land application, which allows biogas production and nutrient re-cycling by means of digestate use as organic fertilizer. However, SS conversion during AD is limited by the low hydrolysis rate of solids and complex organic compounds, which in turn hinders biogas production and the overall organic matter removal during the process (Neumann et al., 2016). Similarly, the degradation of OMP is also limited during AD, as most pharmaceuticals, industrial additives, fragrances and other synthetic compounds present low biodegradability as a result of the high stability of most of the commercially available substances (Oller et al., 2011). The associated risk of contamination and potential human exposure to the OMP present in SS digestate therefore restricts its use as soil conditioner or fertilizer, and novel strategies become necessary to decrease their concentration and ensure the safety of its management.

One of the most extended methods to improve AD hydrolysis and its overall performance is the use of pre-treatments. Pre-treatments imply the use of physical, chemical or biological processes in single or combined configurations in order to hydrolyze SS previous to AD, increasing biogas production and the performance of the stabilization process (Carrère et al., 2010). Most pre-treatment studies have been oriented to biogas production improvements, which has been observed to be related to the efficiency of solubilization achieved through thermal hydrolysis, cavitation, oxidation and other phenomena depending on process configuration (Devlin et al., 2011; Neumann et al., 2018, 2016).

However, solubilization can also increase the bioavailability of OMP to the anaerobic microorganisms, increasing their degradation rate during the AD process. Previous reports indicate that co-metabolism is an important OMP degradation mechanism during AD, which depends on the availability of the specific compounds and therefore the equilibrium between the particulate and soluble phases of sludge (Barret et al., 2010; Delgadillo-Mirquez et al., 2011). Similarly, Aemig et al. (2016) observed that the distribution of PAHs in the different fractions of sludge organic matter greatly influence their degradation during AD, suggesting that hydrolysis processes can be used as a strategy to improve OMP degradation through their transfer to the more accessible phase of sludge. Table 1 shows the results of some previous studies regarding the influence of different pre-treatment processes over OMP removal during AD.

In this work, the influence of ultrasound (US) and thermal (TT; 55°C) pre-treatments over the presence of 10 hydrophobic OMP in mixed SS samples was assessed, mainly selected because of their ubiquity and high frequency of detection (Jones-Lepp and Stevens, 2007). The removal of those compounds in conventional and advanced (i.e. including pre-treatment) AD reactors was also assessed, including a sequential US – TT process which has been reported previously to positively influence AD performance without affecting process stability (Neumann et al., 2018, 2017).

Table 1. Reported effect of some pre-treatment processes over OMP degradation during sewage sludge AD

Studied OMP	Pre-treatment process	Main effects on removal	Reference
Naphthalene; Pyrene	Ultrasound (20 kHz; 0 to 15,000 kJ/kg TS)	*Removal efficiency* Naphthalene: 24.19 ± 1.7 % Pyrene: 11.13 ± 2.4 %	Benabdallah El-Hadj et al. (2007)
Linear alkylbenzenesulphonates (LAS)	Ultrasound (200 kHz)	*Removal efficiency* LAS: 17 – 42 %	Gallipoli & Braguglia (2012)
		Removal efficiency LAS: 38 – 50 % F/I ratios of 0.3 favors the removal of LAS during AD	Gallipoli et al. (2014)
diethyl phthalate; dibutyl phthalate; diethylhexyl phthalate esters	Thermal (70°C) Enzymatic (commercial lipase; 28°C)	*Removal efficiency* <30% Thermal pre-treatment did not had a positive effect over removal, while the enzymatic process was very efficient in the removal of the studied OMP	Gavala et al. (2004)
Galoxolide; Tonalide; Carbamazepine; Diazepam; Ibuprofen; Diclofenac; Iopromide Sulphamethoxazole; Estrone; 17β – estradiol; 17α – ethinylestradiol	Ozonation (20 mg O_3/g TSS; ~2h)	*Removal efficiency* 20 – 99% Removal of carbamazepine was only observed during thermophilic AD after ozonation	Carballa et al. (2007)
12 PAHs, including phenanthrene, anthracene, fluoranthene, pyrene, chrysene and related compounds	Ozonation (0.1 g O_3/gTS)	*Removal efficiency* 20– 63% PAH removal was related to their solubility Efficiency in PAH removal was improved an average of 68% by sludge pre-ozonation, with or without tyloxapol (surfactant) addition	Bernal-Martinez et al (2007)

F/I: Food-to-inoculum ratio

2. Materials and methods

2.1. Sludge sampling

Mixed sludge samples (MSS) were obtained from the Bío-Bío sewage treatment plant in South-Central Chile (36° 48' S, 73° 08' W). The plant treats urban and industrial wastewaters generated in the metropolitan area of Concepción, representing ~500000 inhabitants. Samples were obtained after sludge thickening, transported to the laboratory and stored at 4 °C until analysis. The primary to secondary sludge ratio of the MSS was approximately 40-60% in volume and 65-35% in total solids (TS). Anaerobic inoculum for the AD assays was obtained from the internal recirculation of the 8000 m^3 digesters used in the plant to stabilize the MSS.

2.2. Pre-treatments

2.2.1. Ultrasound (US)

US pre-treatment was performed using a Hielscher UP200St device (Hielscher Ultrasonics GmbH, Germany), at 26 kHz frequency and 200 W power. Briefly, 500 mL of MSS were subjected to US application inside a continuously stirred beaker, with a specific energy (SE) of 2000 kJ/ kg TS. Treatment time varied according to total solids concentration (TS) in the sample, in the range of 2.3 – 6.1 min for a TS of 25.1 – 65.9 g TS/ L. SE was estimated based on Eq. (1).

$$SE\left(\frac{kJ}{kgTS}\right) = \frac{P \times t}{V \times TS} \qquad (1)$$

where P is the power of the US device (200 W), t the pre-treatment time (s), V the sample volume (0.5 L) and TS the total solids concentration (kg/ L).

2.2.2. Thermal treatment (TT)

TT was performed at 55 °C inside a Gerhardt Termoshake THO 500 (Gerhardt GmbH & Co., Germany) orbital stirrer, for 8 h at 70 rpm of continuous agitation. MSS was placed inside 500 mL beakers, covered with performed tops in order to avoid high water evaporation rates. As micro-aerobic conditions have been reported to favor solubilization of sludge in those conditions (Carvajal et al., 2013), plastic hoses were placed within the MSS and connected to the atmosphere.

Solubilization efficiency of pre-treatments (PT) was estimated using the COD solubilization factor (*f*) (Bougrier et al., 2006), corresponding to the ratio between the increase in soluble COD due to pretreatment and the initial particulate COD, as shown in Eq. (2).

$$f\,(\%) = \frac{COD_S - COD_{SR}}{COD_{TR} - COD_{SR}} \times 100 \qquad (2)$$

where COD_S is the COD determined in the soluble phase of the MSS after PT (mg/ L), COD_{SR} is the COD determined in the soluble phase of the raw MSS (mg/ L) and COD_{TR} is the total COD of the raw MSS (mg/ L).

2.3 Anaerobic digestion

Two semi-continuous digesters of 10 L total volume were used during the assays. One of the reactors was fed with raw (non-pretreated) MSS, which constituted the control reactor of the experiment (CR), whereas the other was fed MSS after the sequential application of both PT described in section 2.2 (Pre-treatment reactor, PTR). Both digesters were operated at mesophilic conditions (37 °C) and with a solids retention time (SRT) of 30 d. Further details regarding the digesters set-up and operation can be found elsewhere (Neumann et al., 2018).

2.4 Sludge characterization

Sludge and digestate (biosolids) physicochemical characteristics including chemical oxygen demand (COD), total solids (TS), volatiles solids (VS) were characterized according to Standard Methods (APHA, 2001). Ammonia nitrogen (NH_4^+-N) was determined using a Merck- Millipore Spectroquant® photometric test (2.0–150 mg/L NH_4^+-N) using a UV–VIS Spectroquant® Nova 60/Merck photometer.

2.5 Organic micropollutants (OMP) determination

OMP concentrations were determined in samples of MSS before and after anaerobic digestion. Raw, ultrasonicated and thermally treated MSS samples were assessed, as well as digestate coming from CR and PTR. Results were expressed as µg/g of dry matter.

2.5.1 Chemicals and reagents

n-Hexane, methanol, ethyl acetate, acetone (SupraSolv® quality), casein peptone (triptone), sodium chloride (NaCl) and hexahydrate magnesium chloride ($MgCl_2$ $6H_2O$) were acquired from Merck (Darmstadt, Germany). Triclosan (TCS), tonalide (TNL), galaxolide (GXL), bisphenol A (BFA), butylhydroxytoluene (BHT), butylhydroxyanisole, phenanthrene (PNT), pyrene (PRN), 4–nonylphenol (4NP), tert-octylphenol (TOP), 17-β-ethinylestradiol, 2,2'-dinitrobiphenyl, 10,11–dihydrocarbamazepine and triphenyl amine were acquired from Sigma-Aldrich (Steinheim, Germany). Florisil® and sodium sulfate anhydrous (Na_2SO_4) were acquired from Fluka (Buchs, Switzerland).

2.5.2 Analytical procedure

Molecular structure, main applications and physicochemical properties (molecular weight, log KOW and log KOC) of the studied OMP are provided in Table 2. MSS and digestate were centrifugated for 10 min at 3000 rpm and the solid fraction was liofilized. Briefly, 5 g of the liofilized samples were spiked with a mixture of surrogates (50 ng 2,2'-dinitrobiphenyl for fragrances and 10,11-dihydrocarbamazepine for neutral compounds), they were homogenized in vortex and left to equilibrate for 12 h at 4ºC. Samples were extracted using US with 5 mL of a n-hexane/acetone (1:1, v/v) for 10 min. The extraction was performed three times and the extracts were combined and reduced to ca. 1 mL with nitrogen gas. Extract clean up was performed by adsorption chromatography using 3 g of Florisil® (activated at 400ºC and deactivated with 1% water) and finally eluted with 5 mL ethyl acetate. The extracts were concentrated to ca. 1 mL under a gentle stream of nitrogen and 125 ng of triphenylamine (TPhA) were added as internal standard.

Derivatized extracts were analyzed in a EI-GC-MS/MS Bruker 450-GC gas chromatography coupled to a Bruker 320-MS triple-stage quadrupole mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA).

Table 2. Target organic micropollutants.

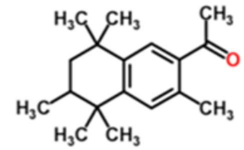
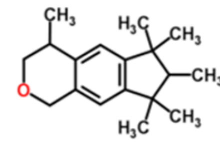
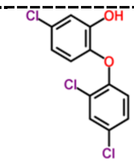
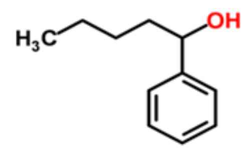
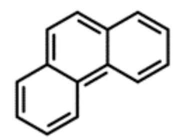
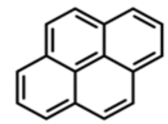
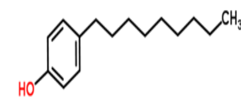
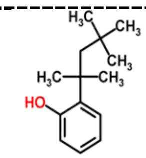
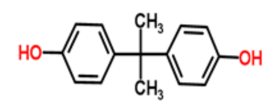
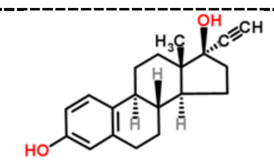
Analyte	Applications	log K_{ow}^{*}	log K_{oc}^{*}	Molecular weight (g mol^{-1})	Molecular Structure	LOD (ng g^{-1})	LOQ (ng g^{-1})
Tonalide (TNL)	Fragrances	6.35	4.72	258.4		65	100
Galaxolide (GLX)		6.26	4.10	258.4		87	148
Triclosan (TCS)	Antibacterial/antifungal	4.66	3.93	289.5		73	121
Butylated hydroxytoluene (BHT)	Antioxidant used in the food, cosmetic and pharmaceutical industries	5.03	3.91	220.4		89	184
Phenanthrene (PNT)	Energy generation	4.35	3.87	178.2		83	108

Table 2. Continued.

Analyte	Applications	log K_{ow}*	log K_{oc}*	Molecular weight ($g\ mol^{-1}$)	Molecular Structure	LOD ($ng\ g^{-1}$)	LOQ ($ng\ g^{-1}$)
Pyrene (PRN)		4.93	4.24	202.3		58	76
4-nonyl phenol (4NP)	Non-ionic surfactant precursor and degradation product	5.99	4.28	220.4		72	97
Tert–octylphenol (TOP)	Starting material for non-ionic surfactant production	5.28	4.01	206.3		101	163
Bisphenol-A (BPA)	Manufacture of plastics and epoxy resins	3.32	3.10	228.3		75	92
17β – ethynyl estradiol (17EE)	Synthetic hormone	4.12	2.71	296.4		72	143

*Log KOW and Log KOC were obtained from EPIsuite v4.00. LOD: limit of detection; LOQ: limit of quantification

The derivatization of samples was carried out by methylation of the acidic hydroxyl groups in a programmed temperature vapolirizing (PTV) injector of the gas chromatograph by adding 10 µL TMSH to a 50 µL samples aliquot before injection. A volume of 5 µL was injected into a Bruker Bruker 450-GC gas chromatography coupled to a Bruker 320-MS triple quadrupole mass spectrometer fitted with a 20 m ×0.18 mm i.d. and 0.18 µm film thickness TRB-5MS capilar column coated with 5% diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugar del Vallès, Sapin). The PTV injector was set at 60ºC for 0.5 min and then rapidly heated up to 300ºC at 200ºC min^{-1} and held for 10 min. Then, the injector was cooled to the initial 60 ºC at 200 ºC min^{-1}. Helium was used as carrier gas (99.9995%) and the flow was selected at 0.6 mL/min. The oven temperature was held at 60ºC for 3.5 min and then the temperature was programmed at 30ºC/min to a 150ºC and finally at 8ºC/min to 320ºC holding the final temperature for 10 minutes. Temperature of the transfer line and ion source were of 250ºC, 200ºC, respectively. Qualitative and quantitative analysis were performed in multiple reaction monitoring mode (MRM), tracking two transitions for every compound as described elsewhere (Reyes-Contreras et al., 2011). Surrogate recovery was 78 ± 4% and 83 ± 7% for fragrances and neutral compounds, respectively. Correlation coefficient (R2) of the calibration curves was in all cases over 0.999.

2.6 Statistical analysis

Statistical significance of OMP concentration after each treatment compared to raw MSS samples was tested by the Wilcoxon signed-rank test (non-parametric test) using Stata software (version 14.2), against a null hypothesis of no difference. A P-value of <0.05 was considered statistically significant.

3. Results and discussion

Table 3 shows the physicochemical parameters of the different MSS and digestate samples. The main effect of pre-treatments (PT) namely US, TT and US+TT was an increase of 250 – 415% in soluble COD concentration (CODs) in the MSS, resulting in 7.8 – 12.9% solubilization factors depending on PT configuration. Moreover, ammonia nitrogen concentration ($N\text{-}NH_4^+$) also showed increases up to 100%, related with both US and TT pre-treatments. However, no effects were observed over total COD (CODt) or solids concentrations (TS, VS).

Table 3. Average concentrations and standard deviation of the physicochemical parameters of sludge and digestate samples.

Parameter	Unit	Raw MSS	MSS after US	MSS after TT	MSS after US – TT	Biosolid after AD	Biosolid after US – TT and AD
COD_t	g/L	45.4 ± 5.0	48.8 ± 5.3	50.9	50.7 ± 5.6	33.8 ± 1.2	32.3 ± 1.7
COD_s	mg/L	1370.9 ± 20.8	4874.7 ± 219.2	4803.8	7057.0 ± 1326.7	2314.3 ± 184.9	2109.8 ± 245.7
CODs/CODt	%	3.02 ± 0.01	10.00 ± 0.03	9.44	13.91 ± 0.07	6.85 ± 0.04	6.54 ± 0.05
f	%	-	7.96 ± 0.04	7.80	12.92 ± 0.09	-	-
TS	g/L	36.5 ± 2.1	35.7 ± 0.1	-	35.9 ± 8.9	23.8 ± 0.6	24.4 ± 1.9
VS	g/L	27.9 ± 0.7	26.7 ± 1.0	-	26.9 ± 5.3	16.1 ± 0.6	15.6 ± 0.4
$N\text{-}NH_4$	g/L	0.2 ± 0.01	0.3 ± 0.01	-	0.4 ± 0.07	0.9 ± 0.06	1.1 ± 0.03

COD: chemical oxygen demand; TS: total solids; VS: volatile solids; N-NH4: ammonia nitrogen; MSS: mixed sewage sludge; US: ultrasound; TT: thermal treatment; AD: anaerobic digestion

Table 4. Average concentration ± standard deviation of the OMP in sludge and digestate samples.

Analyte	MSS			Digestate			
	Raw (µg/g)	US (µg/g)	TT (µg/g)	Conventional AD (µg/g)	Variation* (%)	US – TT and AD (µg/g)	Variation* (%)
Tonalide	0.15 ± 0.04	0.24 ± 0.03	0.17 ± 0.02	0.29 ± 0.06	93.3	0.12 ± 0.05	-20.0
Galaxolide	34.86 ± 6.33	45.66 ± 7.14	34.58 ± 4.23	59.41 ± 9.71	70.4	43.99 ± 8.92	26.2
Triclosan	10.30 ± 3.35	13.37 ± 3.56	10.70 ± 2.91	9.28 ± 3.45	-9.9	2.47 ± 0.52	-76.0
BHT	1.72 ± 0.70	3.28 ± 1.05	2.47 ± 0.55	7.92 ± 2.73	360.5	6.43 ± 2.16	273.8
Phenanthrene	0.26 ± 0.02	0.40 ± 0.03	0.31 ± 0.06	0.48 ± 0.11	84.6	0.33 ± 0.05	26.9
Pyrene	0.14 ± 0.07	0.23 ± 0.04	0.08 ± 0.01	0.24 ± 0.06	71.4	0.19 ± 0.06	35.7
4-nonylphenol	1.34 ± 0.05	1.99 ± 0.22	1.89 ± 0.06	2.45 ± 0.45	82.8	1.45 ± 0.07	8.2
4-Tert-octylphenol	0.32 ± 0.05	0.25 ± 0.07	0.32 ± 0.06	0.94 ± 0.19	193.8	1.17 ± 0.24	265.6
Bisphenol-A	0.22 ± 0.16	2.40 ± 0.21	0.24 ± 0.02	0.29 ± 0.09	31.8	0.67 ± 0.12	204.5
17β–Ethinylestradiol	< LOD	< LOD	< LOD	< LOD	N/A	< LOD	N/A

N/A: Not applicable . * Compared to raw sludge; MSS: mixed sewage sludge; US: ultrasound; TT: thermal treatment; AD: anaerobic digestion

Whereas conventional AD led to removals of 25.6, 34.8 and 42.3% in CODt, TS and VS concentrations, respectively, AD after the sequential pre-treatment showed removals of 28.9, 33.1 and 44.1% in the same parameters. Although PT resulted in a 20% increased concentration of N-NH_4^+ inside the digester, the observed values in both CR and PTR were under the inhibitory threshold reported in literature (Chen et al., 2008; Yenigün and Demirel, 2013). During conventional AD, an increase of 127% in the CODs/CODt ratio was observed in digestate compared to raw MSS, related to the biological hydrolysis and solubilization of organic matter that occurs during the first steps of AD (Gujer and Zehnder, 1983). However, digestate coming from both digesters showed similar CODs values.

Table 4 shows the concentration of the measured OMP in sludge and digestate samples. 9 of the 10 studied compounds were detected in the samples, with the exception of 17EE. PT processes led to increases of 13 – 991% in the concentration of all OMP except 4-tert–octylphenol, most likely related to water evaporation during PT application. Moreover, as US led to higher increases (30 – 991%) compared to TT (4 – 44%), this effect could be at least partially attributed to a positive influence of this PT over the extraction and recovery procedure of OMP from MSS, as US is one of the techniques used for OMP extraction from sludge matrices (Zuloaga et al., 2012).

AD resulted in up to 360% increased concentrations for most of the studied OMP in MSS, with removal being only achieved for TNL (in the pretreated-sludge digester) and TCS (in the conventional and pretreated-sludge digesters). Figure 1 summarizes the influence of both digestion processes over the concentration of OMP, expressed as the ratio between the output (digestate; C) and input (raw MSS; C_0) concentrations of the systems. This result is in contradiction with previous reports, which show that while AD in unable to fully degrade most OMP from sludge, removals of 10 – 99% can be achieved depending on OMP physicochemical properties and operational conditions of digestion (Benabdallah El-Hadj et al., 2007; Bernal-Martinez et al., 2007; Carballa et al., 2007; Gallipoli et al., 2014; Gallipoli and Braguglia, 2012; Gavala et al., 2004). The observed accumulation of OMP inside the digesters could be related to its low biodegradation compared to most of the organic matter that composes MSS, as the transformation of biodegradable organic matter could led to increased concentration of non-biodegradable compounds during AD. However, the increases in OMP concentration surpassed the expected increases based on solids removal efficiencies during digestion (Table 3), and therefore other accumulation mechanisms must be involved in the observed effect.

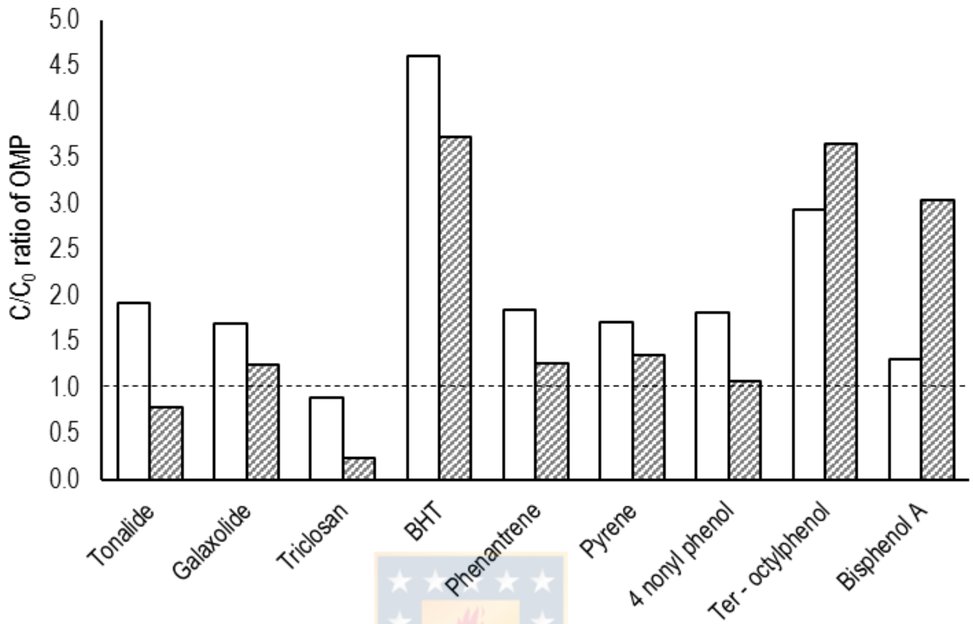


Figure 1. Ratio of digestate (C) to raw MSS (C_0) concentration of the assessed OMP for the conventional digestion (□) and pretreated sludge digestion (▨) processes.

Table 5 shows the results of the non-parametric statistical tests performed over OMP concentrations in the different samples. Statistical analysis between treatments showed some significant differences. However, when P-values were adjusted for multiple comparisons, these differences have not been confirmed, and only some trends are observed. In order to confirm this hypothesis, additional measurements would be necessary.

Table 5. P-values for Wilcoxon test of OMP after each treatment compared to the raw MSS. Shaded cells indicate significant differences between the samples.

OMP	Raw MSS vs AD digestate	Raw MSS vs US sludge	Raw MSS vs TT sludge	Raw MSS vs US – TT and AD digestate
Galaxolide	0.0495	0.2752	0.8273	0.2752
Tonalide	0.0495	0.0495	0.5127	0.2752
Triclosan	0.2752	0.5127	0.8273	0.0495
4-nonylphenol	0.8273	0.2752	0.8273	0.0495
Phenanthrene	0.0495	0.0495	0.2752	0.1266
Pyrene	0.2752	0.1266	0.5127	0.8273
BHT	0.0463	0.1212	0.2683	0.0463
Bisphenol-A	0.8273	0.0495	0.5127	0.0495
4-Tert-octylphenol	0.0495	0.2752	0.8273	0.0495

MSS: mixed sewage sludge; US: ultrasound; TT: thermal treatment; AD: anaerobic digestion

Figure 2 shows the variation in the digestate concentration of OMP caused by the sequential pre-treatment over the different assessed compounds. PT caused reductions in the OMP concentrations of 73% for TCS, 59% for TNL, 41% for 4NP, 19% for BHT, 31% for PNT, 26% for GLX and 21% for PRN. On the other hand, PT resulted in an increased digestate concentration of BPA and TOP of 131% and 25%, respectively.

BPA and TOP are in the low and medium range of Koc for the studied OMP, indicating compounds with comparatively lower hidrophibicities and affinities to the particulate fraction of sludge. During AD, the fate of OMP is mainly goberned by its sorption and biodegradation, as volatilization to the gaseous phase results negligible (Alvarino et al., 2014). Biodegradation is also directly related to sorption, as limited bioavalaibility of OMP hinders its biotransformation. While it has been observed that a fraction of OMP sorbed to particles can indeed be available for degradation (Fountoulakis et al., 2006) and that desorption partially occurs during AD (Macherzyński et al., 2014), PTs could increase their overall bioavailavility through improved transfer of strongly linked compounds from the sludge matrix to the soluble phase (Aemig et al., 2016). As factors that increase the linking of OMC to sludge such as ageing and high hydrophobicity can decrease its biotransformation (Barret et al., 2010; Gonzalez-Gil et al., 2016), it is expected that the use of PTs will have a higher influence over the biodegradation and removal of sequestered or highly hydrophobic compounds during AD.

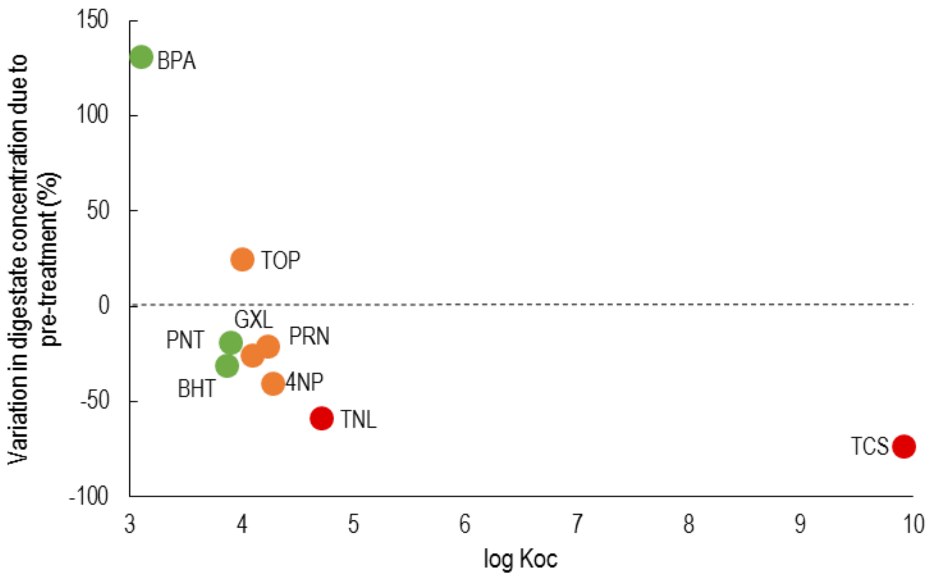


Figure 2. Variation in the OMP concentration in digestate associated with the implementation of the sequential pre-treatment. Negative values represent decreases in the OMP concentration, while positive values represent increases. OMP were classified in three groups according to their log K_{OC}: Red: High (>4.5); Orange: Medium (4.0 – 4.5); Green: Low (<4.0).

4. Conclusion

The influence of different PT configurations over the presence and anaerobic transformation of 10 OMP in mixed sewage sludge (MSS) was assessed. The main effect of the different PT configurations over MSS properties was the solubilization of CODs (7.8 – 12.9% solubilization factors depending on configuration) and increased ammonia concentration (up to 100%). Conventional AD led to removals of 25.6, 34.8 and 42.3% in CODt, TS and VS concentrations, respectively, whereas AD after sequential US – TT PT showed removals of 28.9, 33.1 and 44.1% in the same parameters.

A total of 9 OMP were detected in MSS samples. Conventional and pretreated sludge AD resulted in the accumulation of OMP in the digestate, and only TCS was removed in all the studied conditions. The sequential US – TT-PT assessed in this work resulted in decreased concentrations of 7 of the 9 detected compounds in digestate compared to conventional AD (19 to 73%), with BPA and ter–octylphenol showing the opposite effect. Overall, the results suggest that the assessed PT could mainly improve the removal of sequestered or highly hydrophobic compounds through their solubilization and increased bioavailability.

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References

Aemig, Q., Chéron, C., Delgenès, N., Jimenez, J., Houot, S., Steyer, J.P., Patureau, D., 2016. Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion. Waste Manag. 48, 389–396. https://doi.org/10.1016/j.wasman.2015.11.045

Alvarino, T., Suarez, S., Lema, J.M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. J. Hazard. Mater. 278, 506–513. https://doi.org/10.1016/j.jhazmat.2014.06.031

APHA, A.P.H.A., 2001. Standard methods for the examination of water and wastewater.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781. https://doi.org/10.1016/j.pecs.2008.06.002

Barret, M., Carrère, H., Delgadillo, L., Patureau, D., 2010. PAH fate during the anaerobic digestion of contaminated sludge: Do bioavailability and/or cometabolism limit their biodegradation? Water Res. 44, 3797–3806. https://doi.org/10.1016/j.watres.2010.04.031

Benabdallah El-Hadj, T., Dosta, J., Márquez-Serrano, R., Mata-Alvarez, J., Mata-Álvarez, J., Mata-Alvarez, J., 2007. Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal. Water Res. 41, 87–94. https://doi.org/10.1016/j.watres.2006.08.002

Bernal-Martinez, A., Carrère, H., Patureau, D., Delgenès, J.-P.P., 2007. Ozone pre-treatment as improver of PAH removal during anaerobic digestion of urban sludge. Chemosphere 68, 1013–9. https://doi.org/10.1016/j.chemosphere.2007.02.019

Bougrier, C., Albasi, C., Delgenès, J.P., Carrère, H., 2006. Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability. Chem. Eng. Process. Process Intensif. 45, 711–718. https://doi.org/10.1016/j.cep.2006.02.005

Carballa, M., Manterola, G., Larrea, L., Ternes, T., Omil, F., Lema, J.M.M., 2007. Influence of ozone pre-treatment on sludge anaerobic digestion: Removal of pharmaceutical and personal care products. Chemosphere 67, 1444–1452. https://doi.org/10.1016/j.chemosphere.2006.10.004

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J.J., Delgenès, J.P.P., Steyer, J.P.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: a review. J. Hazard. Mater. 183, 1–15. https://doi.org/10.1016/j.jhazmat.2010.06.129

Carvajal, A., Peña, M., Pérez-Elvira, S., 2013. Autohydrolysis pretreatment of secondary sludge for anaerobic digestion. Biochem. Eng. J. 75, 21–31. https://doi.org/10.1016/j.bej.2013.03.002

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Delgadillo-Mirquez, L., Lardon, L., Steyer, J.P., Patureau, D., 2011. A new dynamic model for bioavailability and cometabolism of micropollutants during anaerobic digestion. Water Res. 45, 4511–4521. https://doi.org/10.1016/j.watres.2011.05.047

Devlin, D.C., Esteves, S.R.R., Dinsdale, R.M., Guwy, A.J., 2011. The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresour. Technol. 102, 4076–82. https://doi.org/10.1016/j.biortech.2010.12.043

EPA, 1995. Land Application of Sewage Sludge and Domestic Septage: Process Design Manual. Environmental Protectio Agency (US-EPA).

Fountoulakis, M.S., Stamatelatou, K., Batstone, D.J., Lyberatos, G., 2006. Simulation of DEHP biodegradation and sorption during the anaerobic digestion of secondary sludge. Water Sci. Technol. 54, 119–128. https://doi.org/10.2166/wst.2006.533

Gallipoli, A., Braguglia, C.M., 2012. High-frequency ultrasound treatment of sludge: Combined effect of surfactants removal and floc disintegration. Ultrason. Sonochem. 19, 864–871. https://doi.org/10.1016/j.ultsonch.2011.12.014

Gallipoli, A., Gianico, A., Gagliano, M.C., Braguglia, C.M., 2014. Potential of high-frequency ultrasounds to improve sludge anaerobic conversion and surfactants removal at different food/inoculum ratio. Bioresour. Technol. 159, 207–214. https://doi.org/10.1016/j.biortech.2014.02.084

Gavala, H.N., Yenal, U., Ahring, B.K., 2004. Thermal and enzymatic pretreatment of sludge containing phthalate esters prior to mesophilic anaerobic digestion. Biotechnol. Bioeng. 85, 561–7. https://doi.org/10.1002/bit.20003

Gonzalez-Gil, L., Papa, M., Feretti, D., Ceretti, E., Mazzoleni, G., Steimberg, N., Pedrazzani, R., Bertanza, G., Lema, J.M., Carballa, M., 2016. Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge? Water Res. 102, 211–220. https://doi.org/10.1016/j.watres.2016.06.025

Gujer, W., Zehnder, a J.B., 1983. Conversion Processes in Anaerobic Digestion. Water Sci Technol 15, 127–167.

Jones-Lepp, T.L., Stevens, R., 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: The interface between analytical chemistry and regulation. Anal. Bioanal. Chem. 387, 1173–1183. https://doi.org/10.1007/s00216-006-0942-z

Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., Jorgensen, G.R., 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. 40, 7207–7215. https://doi.org/10.1021/es0603406

Kupper, T., Berset, J.D., Etter-Holzer, R., Furrer, R., Tarradellas, J., 2004. Concentration and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland RID A-4580-2011. Chemosphere 54, 1111–1120. https://doi.org/10.1016/j.chemosphere.2003.09.023

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Macherzyński, B., Włodarczyk-Makuła, M., Nowacka, A., 2014. Desorption of PAHs from solid phase into liquid phase during co-fermentation of municipal and coke sewage sludge. Desalin. Water Treat. 52, 3859–3870. https://doi.org/10.1080/19443994.2014.887450

Maria, I.C. De, Chiba, M.K., Costa, A., Berton, R.S., 2010. Sewage sludge application to agricultural land as soil physical conditioner. Rev. Bras. Ciência do Solo 34, 967–974. https://doi.org/10.1590/S0100-06832010000300038

Martín, J., Camacho-Muñoz, M.D., Santos, J.L., Aparicio, I., Alonso, E., 2012. Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. J. Environ. Manage. 102, 18–25. https://doi.org/10.1016/j.jenvman.2012.02.020

Neumann, P., Barriga, F., Álvarez, C., González, Z., Neumann, P., Barriga, F., Álvarez, C., González, Z., Vidal, G., 2018. Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound – thermal (55 ° C) pre- treatment. Bioresour. Technol. 262, 42–51. https://doi.org/10.1016/j.biortech.2018.03.057

Neumann, P., González, Z., Vidal, G., 2017. Sequential ultrasound and low-temperature thermal pretreatment: Process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. Bioresour. Technol. 234, 178–187. https://doi.org/10.1016/j.biortech.2017.03.029

Neumann, P., Pesante, S., Venegas, M., Vidal, G., 2016. Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Rev. Environ. Sci. Bio/Technology 15, 173–211. https://doi.org/10.1007/s11157-016-9396-8

Oller, I., Malato, S., Sánchez-Pérez, J.A., 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination-A review. Sci. Total Environ. 409, 4141–4166. https://doi.org/10.1016/j.scitotenv.2010.08.061

Reyes-Contreras, C., Matamoros, V., Ruiz, I., Soto, M., Bayona, J.M., 2011. Evaluation of PPCPs removal in a combined anaerobic digester-constructed wetland pilot plant treating urban wastewater. Chemosphere 84, 1200–1207. https://doi.org/10.1016/j.chemosphere.2011.06.003

Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochem. https://doi.org/10.1016/j.procbio.2013.04.012

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M., Prieto, A., 2012. Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: a review. Anal. Chim. Acta 736, 7–29. https://doi.org/10.1016/j.aca.2012.05.016

CAPÍTULO V

PRESENCE AND FATE OF MICROPOLLUTANTS DURING ANAEROBIC DIGESTION OF SEWAGE SLUDGE AND THEIR IMPLICATIONS FOR THE CIRCULAR ECONOMY: A MINI-REVIEW

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Presence and fate of micropollutants during anaerobic digestion of sewage sludge and their implications for the circular economy: a mini-review

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Abstract

Circular economy (CE) is defined as a system in which the value of products, materials and resources is maintained for as long as possible, minimizing their consumption and the generation of waste. Within the CE framework, anaerobic digestion (AD) represents an attractive technology, as it uses waste to produce biogas as renewable energy and stabilizes the sewage sludge for land application. In this way, this technology contributes to "closing the loop" between energy consumption, food production and the disposal of the subsequent waste. These potential benefits may be limited by negative impacts related to the land disposal of the stabilized sewage sludge. For example, the presence of micropollutants (MPs) in the input sewage and the inability of current AD methods to remove them from the stabilized sewage are recognized potential risks for human health and for the environment. The aim of this mini-review is to provide an overview of the different MPs present in the raw sewage sludge and stabilized sewage sludge (biosolids) with AD, to assess their potential adverse effects, and to highlight possible remediation strategies. This review will focus on three important groups: pharmaceuticals and personal care products (PPCPs), metallic trace elements, and polycyclic aromatic hydrocarbons (PAHs). The resulting toxicity of the biosolids will

depend on the operational characteristics of AD and on the physicochemical properties of the different MPs. These two factors ultimately determine their final concentration, their persistence and bioaccumulative potential, and the formation of metabolites, which sometimes can be more toxic than the corresponding parental compounds.

1. Introduction

Sewage sludge generation has increased in the last decades due to the introduction of aerated technologies (i.e., activated sludge) in sewage treatment. As a consequence, the accumulation of non-stabilized sludge generates environmental problems for populations and ecosystems. Sewage sludge is characterized by high concentrations of solids and organic matter, with a significant presence of pathogens, including antibiotic resistant bacteria (Neumann et al. 2016). In addition, it contains a variety of micropollutants (MPs), like pharmaceuticals, hormones, pesticides, and household and industrial chemicals (Gonzalez-Gil et al. 2018), which may constitute a risk for environmental and human health.

Anaerobic digestion (AD) is a widely used process for sewage sludge stabilization, in which the organic matter is broken down by a consortium of microorganisms under anaerobic conditions (Chen et al. 2008). During AD, biogas is produced as a mixture of gaseous compounds, principally methane (CH4) and carbon dioxide (CO2), via volatile fatty acid (VFA) degradation (Zhang et al. 2016). Moreover, the organic matter is transferred to a semisolid phase during the treatment, constituting the so-called stabilized sewage sludge or biosolids (Neumann et al. 2017, 2018).

These two resources, biogas and the biosolids, constitute valuable resources under the concept of circular economy (CE). CE is defined as an alternative to the traditional economy in which the value of products, materials and resources is maintained for as long as possible, therefore minimizing waste production and resource consumption (Malinauskaite et al. 2017). The need to transition to CE is recognized as an essential element in developing a sustainable, resource-efficient and competitive economy. In this context, the biogas produced in DA represented a commercial value as a bioenergy source (Appels et al. 2008). Likewise, the biosolids from DA offers a sustainable waste treatment strategy that combines waste stabilization and nutrient recovery, and it can be used as biofertilizer for soil application with beneficial effects (Abdullahi et al. 2008).

The use of stabilized sewage sludge for agricultural purposes is an attractive solution for enhance the soil fertility because it increases soil organic C storage, improves soil aeration and favors cation exchange (Mattana et al. 2014). However, the presence of pollutants, like heavy metals, organic pollutants and pathogens, represents potential risks for the environment and human health (Alvarenga et al. 2016). An active line of research is currently being developed to study the presence, concentrations, fate and potential negative effects of MPs found in sewage sludge and biosolids (Gao et al. 2012; Martín et al. 2015a; Stasinakis et al. 2012; González-Gil et al. 2016). The objective of this mini-review is to provide an overview of the different MPs present during the AD of sewage sludge, their associated risks and the possible remediation measures, under the framework of the circular economy.

2. Bibliometric analysis

For the bibliometric search, Scopus and Web of science database were used. The search parameter was anaerobic digestion, sewage sludge, and biosolid. These parameters were associated with their appearance in the article title, abstract, and keywords. From this search, 9761 articles were found. Subsequently, a process of data depuration was carried out. Any article outside the objectives of this work was eliminated. At the end of this process, the data set was 3168 articles from the year 1947 until 2019. From the initial data set, a new data set was elaborated considering the articles in the micropollutants category. The software VOS viewer 1.6.11 (CWTS 2019), was used for construct and visualizing the bibliometric data set, considering the co-occurrence networks of important terms extracted from the title and abstract fields in the data sets.

2.1. General bibliometric analysis

Figure 1 shows the distribution of the 3168 articles in five topics considering the investigation of the behaviors of organic matter, micropollutant, heavy metals, pathogens and nutrients. The predominant topic in the data set was the behaviors of organic matter with 64% of the articles, that mainly considered the pre-treatment and operational parameter influence on anaerobic digestion development. Followed by 15% of the articles in the data set that investigate the behaviors of the micropollutant during anaerobic digestion of sewage sludge. Finally, with 6, 7, and 8% of the articles in the data set are researches that analyzed the behaviors of pathogens, nutrients, and heavy metals, respectively.

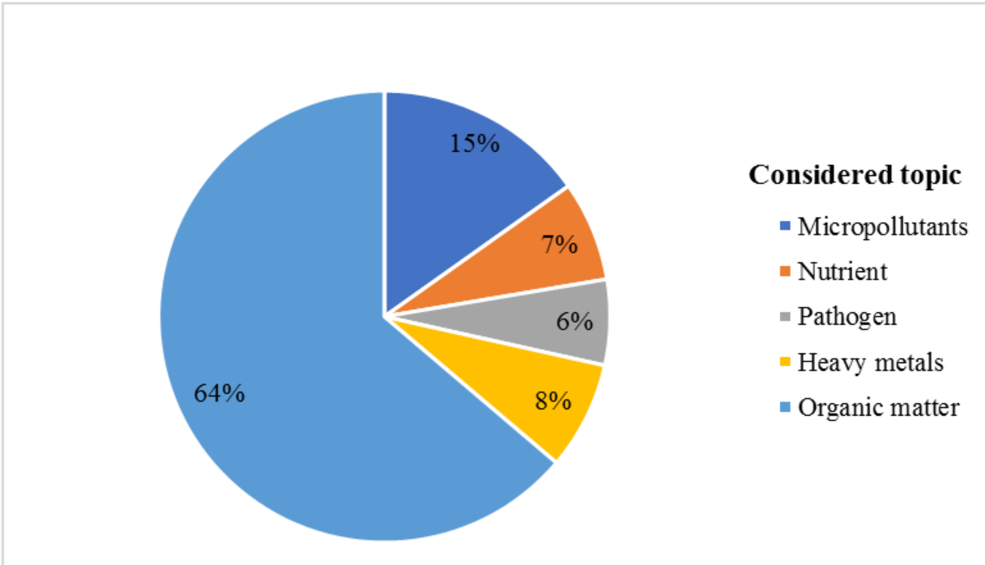


Figure 1. Percentage distribution of topics related to the behaviors of organic matter, micropollutants, heavy metals, nutrients and pathogens in the anaerobic digestion of sewage sludge.

There is a gradual increase in the number of publications that study other issues besides the performance of anaerobic digestion of sewage sludge (64% of the data set, Figure 1), where the research that considers the behaviors of heavy metals, nutrients and pathogens have maintained similar numbers of publications over time (Figure 2). On the other hand, since 2000-2009 the number of publications related to the behaviors of micropollutants has increased considerably, reaching nearly 100 publications for that period, while the other topics have less than 50 publications for the same period. Trend that is more marked in the last 10 years, where the number of publications related to micropollutants exceeds 300, while in the other subjects does not exceed 100 publications.

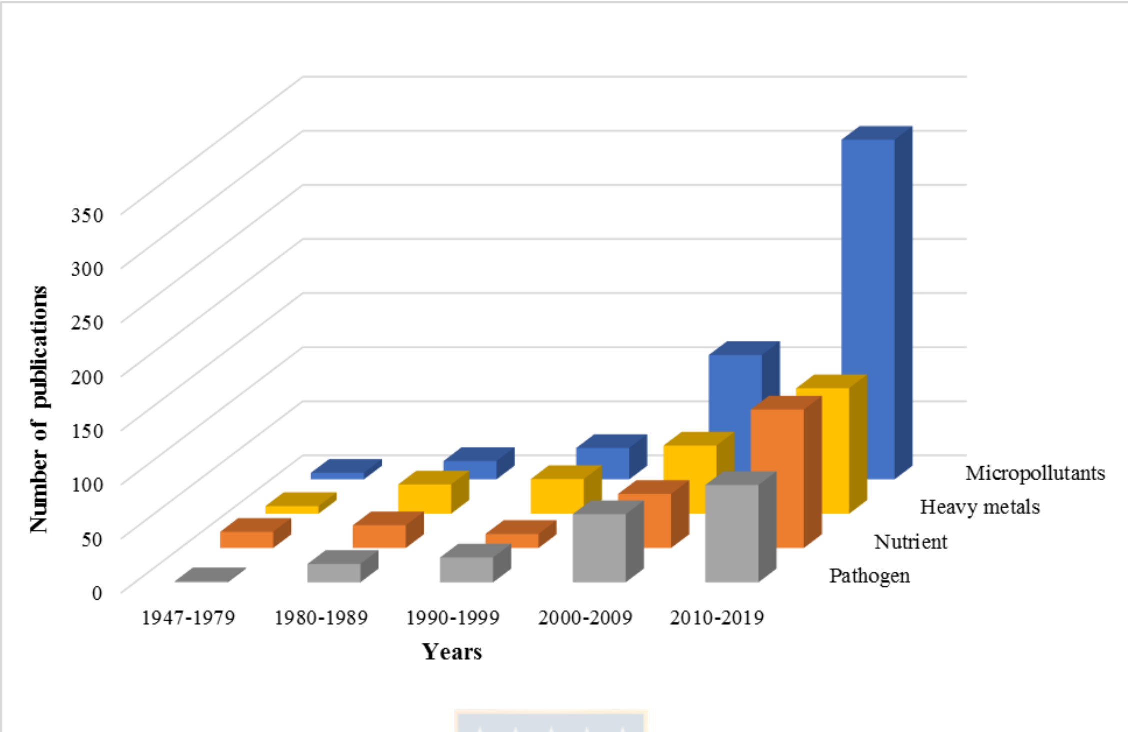


Figure 2. Temporal trend of topics related to the behaviors of micropollutants, heavy metals, nutrients and pathogens in the anaerobic digestion of sewage sludge.

2.2. Co-occurrence network analysis

Figure 3 shows a general visualization considering the co-occurrence networks of terms extracted from the title and abstract fields in the data sets of micropollutant topics. Figure 3a shows a general visualization of all the terms grouped in six clusters. It can be seen some general terms as sewage treatment, country, analyte, WAS (wasted activated sludge), waste, anaerobic fermentation, digestate. This general term overshadows other more specific and interesting to analyze. So, Figure 3b showed a specific co-occurrence network with eight clusters (colors in Figure 3a are not related to color in Figure 3b). Besides, Figure 3c shows the same cluster from 3b but colored by publication year, this show how a contaminant or term of interest trend with the passage of the time.

The red cluster (Figure 3b) are related with terms as antibiotic resistance bacterium and genes, where the principal genes where inti1 (integron), sul1 (sulfonamide-resistance gene), and tetracycline resistance genes (tetX, tetG, tetA, tetO). The occurrence of these terms has been stronger in the last five years (Figure 3c). The green cluster (Figure 3b) grouped xenobiotic compound as dioxins and furans (PCDD/F), and phthalates compounds as phthalic acid esters (PAE), dibutyl phthalate (DBP), di-(2-ethyhexyl) phthalate) (DEHP), and polychlorinated biphenyl (PCB), where the occurrence of these terms was stronger before 2006 (Figure 3c). The purple cluster (Figure 3b) consider polycyclic aromatic hydrocarbons (PAH, PAHs) principality, with a temporal occurrence between 2006-2008 (Figure 3c).

Furthermore, the cyan cluster (Figure 3b) consider generic term as trace organic compounds (TROC, TROCs) and two pharmaceutical compound caffeine and paracetamol, a stimulant and an analgesic, respectively. With a temporal occurrence more frequent in the last five years (Figure 3c). Another cluster with a temporal frequency stronger in this same period (Figure 3c) is the brown one (Figure 3b) which group nano-compound related term as engineered nanomaterials (ENM), and a nanoparticle, as well as nano-molecules as zinc oxide (ZnO). On the other hand, the orange cluster (Figure 3b) mainly consider two antibacterial agents: triclosan (TCS) and triclocarban (TCC) with a temporary occurrence between 2010 and 2019.

The last two clusters consider several pharmaceuticals and personal care products (PPCP). The blue cluster (Figure 3b) group synthetic musk and fragrances as galaxolide (HHCB) and tonalide (AHTN); anti-inflammatory drugs as ibuprofen and diclofenac (DCF); anti-epileptic drug as carbamazepine (CBZ), antibiotics as ciprofloxacin and ofloxacin; and other compounds as siloxane, clofibric acid (CFA). The temporary occurrence of this cluster is between 2010-2014 (Figure 3c). The last cluster (yellow, Figure 3b) include four types of compound: surfactant as linear alkylbenzene sulphonates (LAS); anti-inflammatory drugs as ketoprofen and naproxen; estrogens and hormones as estrone (E1), estrogen, and 17a-ethinylestradiol (EE2); and industrial estrogens as bisphenol A, nonylphenol (NPE) compounds: nonylphenol(poly)ethoxylates (NPEO), nonylphenol monoethoxylate (NP1EO), and nonylphenol diethoxylate (NP2EO). Also, this cluster includes general terms as endocrine disrupting compounds (EDC, EDCs)). The temporary occurrence of this cluster varies widely during the time (Figure 3c).

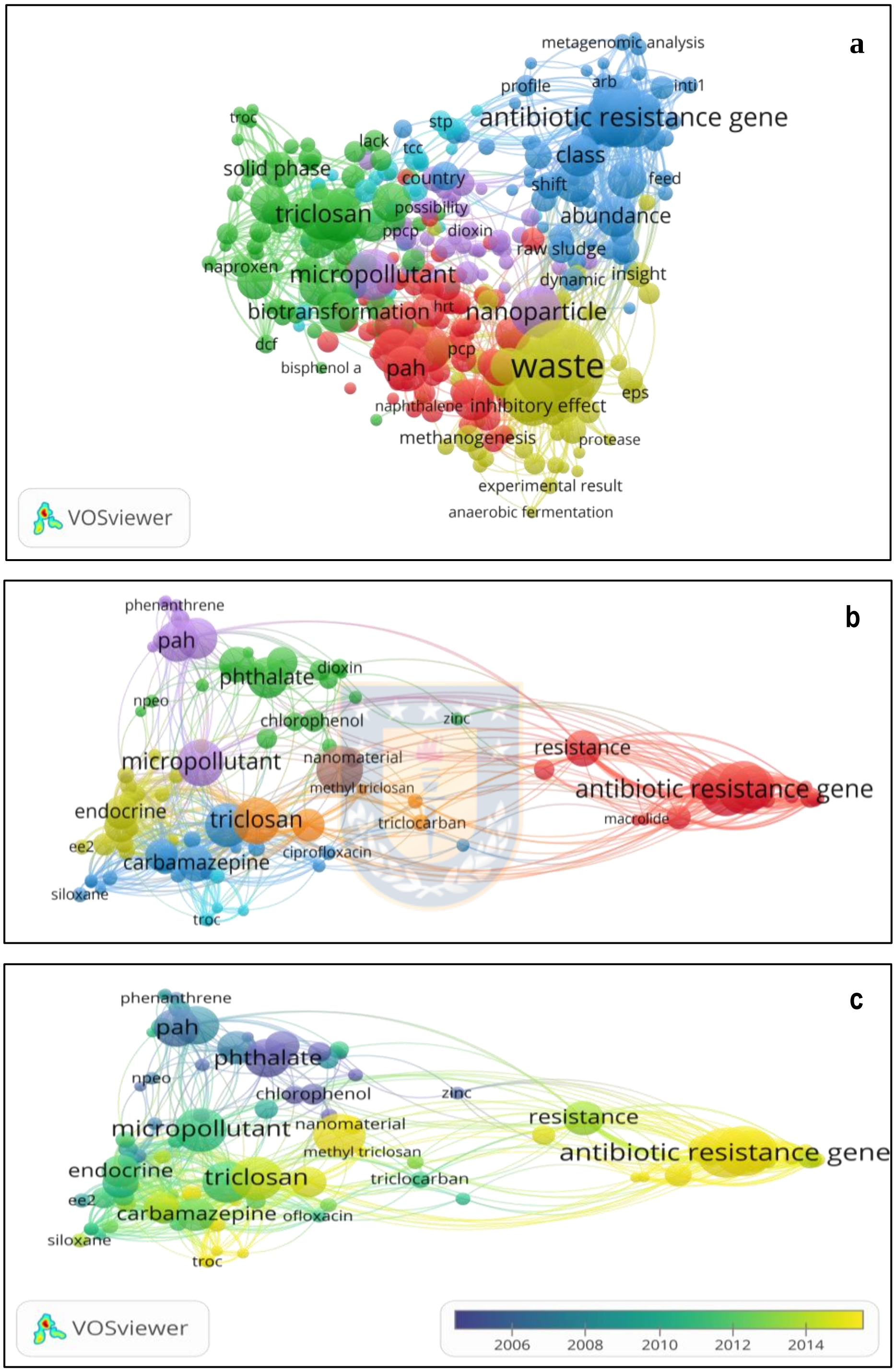


Figure 3. Co-occurrence networks term of the data set considering micropollutant topic. a) general co-occurrence networks considering all term from the data set, b) specific co-occurrence networks term colored by cluster; and c) specific co-occurrence networks term colored by publication year.

3. Micropollutants (MPs)

MPs are anthropogenic or natural compounds present in the environment at trace concentrations ranging from ng/L to µg/L (Kim et al. 2007) for which negative effects on environmental or human health are either confirmed or suspected, even at environmentally relevant low concentrations. This relatively low concentrations of MPs in wastewaters not only complicates their detection and quantitation, but also creates challenges for water and wastewater treatment processes (Luo et al. 2014). As the potential relevance of these substances in the global anthropogenic toxicity has been realized only recently, these compounds are also referred to as Contaminants of Emerging Concern (CEC). These contaminants include pharmaceuticals and personal care products (PPCPs), metallic trace elements, polycyclic aromatic hydrocarbons (PAHs), plasticizers, flame retardants and surfactants. The described toxic effects of MPs cover a wide range of deleterious effects, from DNA damage and mutagenesis to dysfunctions in the endocrine system, reproductive toxicity, immunological impairment or developmental defects, both humans and the wildlife (De Jesus Gaffney et al. 2015; WHO 2006).

The occurrence of MPs in sewage sludge and biosolids from AD has been widely reported (Clara et al. 2011; Kupper et al. 2004; González-Gil et al. 2016; Stasinakis 2012). The concentrations of these compounds in sewage sludge are associated to their physicochemical properties such water solubility, the octanol-water partition coefficient (Log K_{ow}), and soil adsorption coefficient (Log K_{oc}). K_{ow} is frequently used to predict absorption of MPs on solids (Luo et al. 2014). According to Rogers (1996), Log K_{ow}<2.5 indicates low sorption potential, 2.5<Log K_{ow}<4 indicates medium sorption potential and Log K_{ow}>4 indicates high sorption potential. In the same way, K_{oc} is a measure of the tendency of compounds to bind to soils. Higher Log K_{oc} values correlate to less mobile organic chemicals while lower Log K_{oc} values correlate to more mobile organic chemicals (USEPA 2002). Moreover, the sludge characteristics (pH, organic matter, cation's concentration, and others) and the operation parameters of treatment plants, as the hydraulic retention time and organic loading rates (OLRs) also affected the concentration of MPs (Stesinakis et al. 2008).

3.1. Pharmaceuticals and personal care products (PPCPs) in sewage sludge

As an important group of MPs, PPCPs have received growing attention in recent years for their possible negative effects to the environment and human health (Ebele et al. 2017). They contain diverse groups of organic compounds used for the daily personal care, like soaps, lotions, fragrances and sunscreens (Liu et al. 2013). Table 1 shows the physicochemical properties and concentrations of PPCPs detected in sewage sludge and biosolids from AD. Regarding fragrances, the most common compounds were galaxolide and tonalide, with values that varied between 13 to 427000 µg/kg in sewage sludge. These substances have hydrophobic characteristics, with Log K_{ow} values above 6, and therefore found in the solid phase (sewage sludge) rather than in the liquid phase (Clara et al. 2011). Triclosan is the mostly current used in bactericide in toothpaste, detergents and sports clothing (Bester 2003), reaching concentrations of 620 to 17500 µg/kg in secondary sludge and 190 to 9850 µg/kg in biosolids.

Pharmaceutical drugs are also present in both urban and agricultural sewage emissions. The most prevalent compound in sewage sludges is ibuprofen, with values that fluctuate between 1.9 to 950 µg/kg. For this anti-inflammatory compound, Log K_{ow} is ranged between 2.5 and 4, being therefore present in both sludge phases (solid and liquid) (González-Gil et al. 2016). Hormones and hormone-like compounds are also commonly found in sludges, either from natural sources (estriol, estrone and estradiol) or from hormonal regulation pharmaceuticals, like ethynyl estradiol or progestanes (Lai et al. 2002). These compounds are extremely potent toxicants as they are able of interact with the endocrine system, altering it and affecting the reproductive functions of humans and other animals, a toxic effect known as endocrine disruption (ED). The best-known ED activity is the feminization effect of many natural or synthetic estrogens, which are found both in the sewage effluents and sludges, at concentrations high enough to elicit physiological responses in fish and other vertebrates (Céspedes et al. 2004; Chamorro et al. 2010).

A particular category of pharmaceuticals also present in sewage sludge are antibiotics, widely used in both human and livestock antibacterial treatments. Although their toxicity for humans and animals is intrinsically very low, there is a growing concern that their persistence in sludges and soils may favor the emergence of antibiotic resistant bacteria that can ultimately result in untreatable bacterial illnesses in humans or livestock (Piña et al. 2018; Zhang et al. 2009). One example is sulfamethoxazole, found in sewage sludge at concentrations around 100µg/kg, and with a high potential to migrate to the liquid phase, given its Log K_{ow} value of0.89 as shown Table 1 (Gonzalez-Gil et al. 2018).

Table 1. Physical-chemical properties and concentrations of PPCPs detected in sewage sludge.

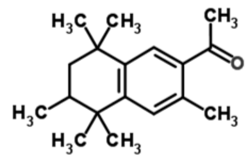
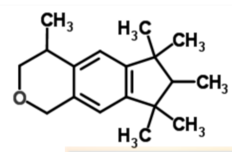
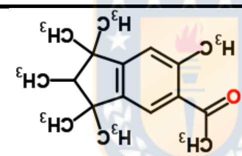
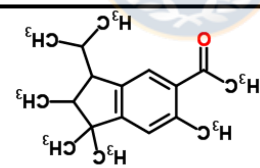
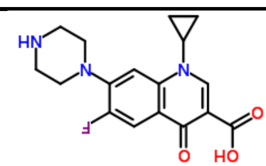
Classification	Analyte	Log K_{ow}	Log K_{oc}	Molecular structure	Matrix	Concentration (µg/kg)	Ref.
Fragances	Tonalide	6.35	4.72		Digested sludge	4000	[1];[2];[3];[4];[5]
					Biosolid	78-427000	
					Secondary sludge	400-11200	
					UASB sludge	803-2407	
	Galaxolide	6.26	4.10		Digested sludge	26000	[1];[2];[3];[4];[5]
					Biosolid	13-177000	
					Secondary sludge	4200-36000	
					UASB sludge	3842-12304	
	Phantolide	5.30	3.64		Secondary sludge	200-1800	[3]
	Traseolide	5.76	3.77		Secondary sludge	200-1000	[3]
Antibiotics	Ciprofloxacin	0.28	1.55		Digested sludge	1000-2400	[6];[7]
					Secondary sludge	960	[8];[9]

Table 1. Continued.

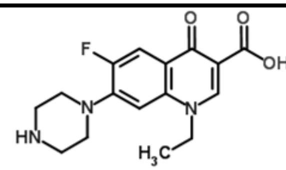
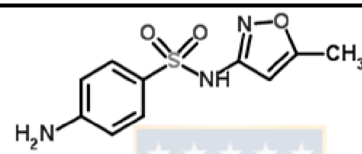
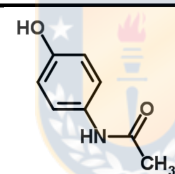
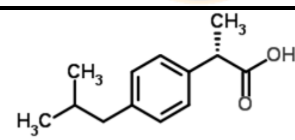
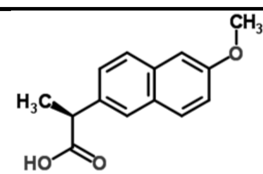
Classification	Analyte	Log K_{ow}	Log K_{oc}	Molecular structure	Matrix	Concentration (µg/kg)	Ref.
Antibiotics	Norfloxacin	-1.03	1.96		Digested sludge	1500-2400	[8]
	Sulfamethoxazole	0.89	3.185		Secondary sludge	113	[10]
Drugs	Acetaminophen	0.46	1.79		Biosolid	1400	[2]
					Secondary sludge	111	[6]
	Ibuprofen	3.97	2.59		Secondary sludge	1.9-950	[11];[12]
	Naproxen	3.18	2.54		Secondary sludge	32.9-50	[13];[7]

Table 1. Continued.

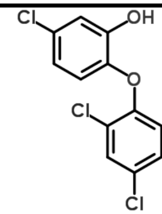
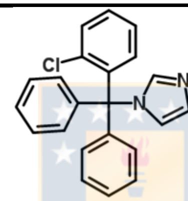
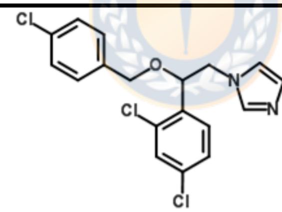
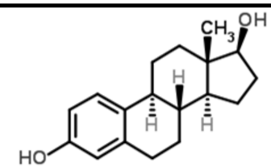
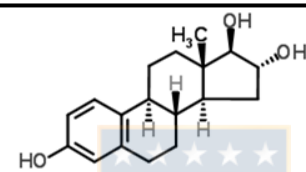
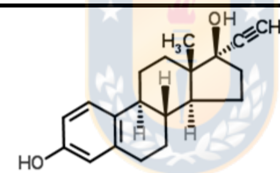
Classification	Analyte	Log K_{ow}	Log K_{oc}	Molecular structure	Matrix	Concentration (µg/kg)	Ref.
Personal Care Products	Triclosan	4.76	4.26		Secondary sludge	620-17500	[14];[15];[16]
					Biosolids	190-9850	[16]
	Clotrimazole	6.26	6.21		Secondary sludge	50	[17]
	Econazole	5.61	4.57		Secondary sludge	210	[17]

Table 1. Continued.

Classification	Analyte	Log K_{ow}	Log K_{oc}	Molecular structure	Matrix	Concentration (µg/kg)	Ref.
Hormones	β-Estradiol	4.01	4.20		Secondary sludge	17-50	[12];[13]
	Estriol	2.45	2.90		Secondary sludge	80	
	Ethinylestradiol	3.67	4.68		Secondary sludge	24-160	

Notes: Kow: octanol-water partition coefficient; Koc: soil adsorption coefficient. Both values were obtained from Chemspider®; **References:** [1]: Stevens et al. (2003); [2]: Kinney et al. (2004); [3]: Kupper et al. (2004); [4]: Clara et al. (2011); [5]: Reyes-Contreras et al. (2011); [6]: Gao et al. (2012); [7]: Martín et al. (2015); [8]: Golet et al. (2002); [9]: Jia et al. (2012); [10]: Göbel et al. (2005); [11]: Martin et al. (2012a); [12]: Martin et al. (2012b); [13]: Heidler et al. (2009); [14]: Chu and Metcalfe (2007); [15]: Peysson and Vulliet (2013); [16]: Stasinakis et al. (2008); [17]: Lindberg et al. (2010)

3.2. Metallic trace elements in sewage sludge

Metallic elements are ubiquitously found as traces in the environment due to natural geological activities. Many of them (Fe, Cu, Zn) are in fact essential micronutrients involved in enzyme activities of organisms (Madigan et al. 2012). However, when concentrations reach excessive values, heavy metals can cause important environmental and human health impacts due to their interactions with molecules of high importance in living organisms (Thanh et al. 2016). Metallic trace elements have an important role in AD performance, a fact that has been traditionally overlooked. Fe and Ni act as cofactors of hydrogenases participating in the acidogenic and methanogenic hydrogenotrophic stages of CH_4 production (Braga et al. 2017). Conversely, excessively high trace metal concentrations may result in AD inhibition (Vignais and Colbeau 2004). Finally, an excessive metal content in the sewage sludge will cause harm to the environment and minimize the quality and applicability of AD products in land disposal (Thanh et al. 2016).

Table 2 shows a summary of concentrations of metallic trace elements in different samples of sewage sludge and biosolids from AD. As observed, all the reported concentrations are below the limits established in international directives, and the sludges can therefore be used in agriculture. The data also show a high variability in the concentrations of environmentally important metals, like Pb or Cr, that can be attributed to the characteristics of the wastewaters that originated the sludge samples, and therefore to variables like city economy, size, industrialization and rain/wastewater management strategies.

Table 2 Concentration of different metallic trace elements in sewage sludge and maximum permissible concentration for agricultural uses according to European Union recommendations.

Metals	Concentration (mg/kg)	EU recommendations (mg/kg)*	Ref.
Al	9900	--	[1]
As	<0.01-3.67	--	[1];[2];[3];[4]
B	23.8-59.58	--	[1];[2]
Be	0.43	--	[3]
Cd	0.94-8.3	40	[1];[2];[3];[4];[5];[6];[7];[8]
Cu	58.7-700	1750	[1];[2];[3];[4];[5];[6];[7];[8];[9]
Cr	13-627	1500	[1];[2];[3];[4];[5];[6];[7];[9]
Fe	10.56-25000	--	[1];[2];[3];[5];[6]
Hg	<0.01-7.97	25	[1];[3];[4];[5]
Mn	98-587	--	[1];[2];[3]
Ni	6.7-239.4	400	[2];[3];[4];[5];[6];[7];[9]
Pb	11.7-800	1200	[1];[2];[3];[4];[5];[6];[7];[8];[9]
Se	21-166.1	--	[3]
Zn	250-1378	4000	[1];[3];[5];[6];[7];[8];[9]

References: [1]: Maria et al. (2010); [2]: Aguilera et al. (2007); [3]: Silva Oliveira et al. (2007); [4]: Tampio et al. (2016); [5]: De Souza Pereira and Kuch (2005); [6]: Garrido et al. (2012); [7]: Gondek et al. (2018); [8]: Villar and García (2006); [9]: Braga et al. (2017).*Directive 86/278/EEC.

3.3. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs) in sewage sludge

PAHs are products of incomplete combustions, with their main source being the burning of biomass and fossil fuels. The structure of these hydrophobic organic compounds contains at least two fused benzene rings. This complex molecular structure conditions their biological degradation (Zhou et al. 2015), and their hydrophobic nature promotes a high affinity for organic phases of soil and specific mineral surfaces (Yang et al. 2013). Table 3 summarizes different studies that reported the occurrence and concentration of these MPs in sewage sludge and biosolids. Paraíba et al. (2010) conducted a study in order to assess the presence of PAHs in soil and sewage sludges and to simulate the long-term risk of soil contamination by PAHs. They found that the maximum concentration of PAHs in sewage sludge from a municipal treatment plant was 2.5 mg/kg. The same values were reported by Wolejko et al. (2018) with concentrations that varied between 2.3-2.6 mg/kg. This study analyses sources of 16 PAHs and determine how sewage sludge changes PAHs content in urbanized areas soils. Exposure to PAHs, either by inhalation or by ingestion, have been associated to a variety of detrimental effects, including cardiotoxicity, mutagenicity, carcinogenesis and respiratory illnesses (Mesquita et al. 2014).

Another group of pollutants that have received attention are polychlorinated biphenyls (PCBs). PCBs are a family of compounds that were extensively used as dielectric and coolant fluids, especially in transformers, capacitors, and electric motors before being banned in 2001 by the Stockholm Convention. Due to their extremely high persistence and hydrophobicity (log K_{ow} values from 4.5 to 8.2), they are found predominantly absorbed on the sludge sediment and bioaccumulate along the trophic chains, reaching extremely high concentrations in top predators (Van der Oost et al 2003). This high bioaccumulative potential is on the root of their multiple negative effects on health, including carcinogenesis and effects on the immune, neurological, endocrine, and reproductive systems (Rosinska and Karwowska 2017). Leiva et al. (2010) studied the mobility and behavior of PCBs in soils amended with biosolids, using spiked anaerobically digested sludge from a disposition site. The concentration of PCBs prior to the external addition of pollutants were around 0.44 and 0.66 mg/kg. Likewise, Rosinska and Karwowska (2017) evaluated the influence of mesophilic AD on degradation of PCB in sewage sludge and on dynamics changes during the process. In this case, concentrations reported were between 0.0016 to 0.023 mg/kg and showed that after the digestion, the concentrations of PCBs decreased 57-90.3%.

Polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs) are two groups of tricyclics, planar aromatic compounds. They are mainly introduced into the biosphere as a by-product of the chemical manufacturing industry or as a result of combustion of organic substances in the presence of chlorinated compounds, like manufacture of chlorophenols, pulp chlorination and incineration of wastes (Muñoz et al. 2018). While some PCDD/Fs have been shown to form during wastewater treatment processes, this is considered as a minor source, insignificant compared with inputs via the sludge itself (Alcock and Jones 1996). De Souza Pereira and Kuch (2005) found concentrations of PCDD/Fs of 1313 pg/g for digested sludge with toxicities (I-TEQ/g) within the range of other studies and concentrations below the upper limit concentration for land disposal, according to German federal legislation for agricultural use (100 pg I-TEQ/g, dry weight).

Table 3 Summary of studies reporting concentration of organic micropollutants in sewage sludge.

Pollutant	Matrix studies	Concentration	Objective of the study	Ref
PAHs	Sewage sludge from municipal WWTP.	<2.5 mg/kg	To assess the presence of PAHs in soil and sewage sludge and to simulate a long-term risk of soil contamination by PAHs.	[1].
	Sewage sludge from the WWTP of 4 SBR.	2.3-2.6 mg/kg	To determine the sewage sludge influence on changes in PAHs contents in urbanized areas.	[2]
PCBs	Anaerobically digested sludge forms a monofill with and without external addition of PCBs.	For samples without PCBs: 0.44-0.66 mg/kg For samples with PCBs: 535-574 mg/kg	To assess the mobility and behavior of PCBs in soils amended with sewage sludge.	[3]
	Digested sludge from a mesophilic anaerobic digestion.	0.0016-0.023 mg/kg	To evaluate the influence of mesophilic anaerobic digestion on the dynamics of changes and the degradation kinetics of PCBs in sewage sludge.	[4]
PCDD/F	Digested sludge from the WTP.	1313 pg I-TEQ/g	To study the concentration of heavy metals and PDDC/PCDF and PCB in different sludge samples.	[5]
	Samples of compost from a composting plant and sewage sludge from WWTPs.	17 pg I-TEQ/g	To deduce the factors that can cause a significant formation of PCDD/F and some explanation to justify the different concentrations of dioxins observed in samples of the same composting plant.	[6]

Notes: I-TEQ: International Toxic Equivalency; SBR: Sequencing Batch Reactor; WWTP: wastewater treatment plants; **References:** [1]: Paraíba et al. (2001); [2]: Wolejko et al. (2018); [3] : Leiva et al. (2010); [4]: Rosinska and Karwowska (2017); [5]: De Souza Pereira and Kuch (2005); [6]: Muñoz et al. (2018).

3.4. Biotransformation of micropollutants (MPs) during anaerobic digestion (AD)

In general, MPs can be removed mainly through processes: biotransformation and adsorption (Liu et al. 2013). It is known that the capacity of AD to remove MPs by biotransformation is limited (Stasinakis 2012). The main factors influencing this biotransformation process were physicochemical properties of MPs, temperature (mesophilic and thermophilic digestion), sludge retention time (SRT) and OLR (González-Gil et al. 2018). Table 4 summarizes the removal efficiencies of different MPs during AD. González-Gil et al. (2016) studied the removal of 20 MPs during mesophilic (37°C) and thermophilic (55°C) AD, also used chemical and biological methods to evaluate the potential risks of MPs. For β-estradiol, galaxolide and triclosan, they were not eliminated during AD (mesophilic and thermophilic) with values below 20%. The biotransformation of ibuprofen was also studied with values close to 30%. Finally, sulfamethoxazole was presented in the group where the compounds were most efficiently removed with percentages above 80%. In this study, the influence of temperature (37°C and 55°C) on the biotransformation of MPs was not significant, except for one compound (17α-ethinylestradiol). Regarding bioassays applied in this research, the different results showed that temperature (55°C) reduced the estrogenic activity in cell line MCF-7 and according to the Ames test, the mutagenic activities were not detected but genotoxic effects were observed in Comet test.

In addition, Braguglia et al. (2015) evaluated the impact of AD with pre-treatment (thermal hydrolysis and ultrasound) operated in thermophilic conditions on the quality of final digested sludges by assessing MPs. The biotransformations of PAHs and PCBs were 33-73% and 70%, respectively. However, the biosolids showed toxicity to *Arthrobacter globiformis*. Furthermore, Butkovskyi et al. (2016) studied the removal of MPs during the composting of upflow anaerobic sludge bed sludge and waste wood. This study achieved removal efficiencies up to 85% for diclofenac, carbamazepine, metoprolol and triclosan. Percentages (12-24%) of this last compound was transformed to methyltriclosan, a persistent by-product having a potential for accumulation in soils. The acute toxicity of this MP was reported by bioluminescence inhibition of *Vibrio fischeri* (Farré et al. 2008).

Other factors that influence the removal of different MPs during AD are microbial population composition and metabolism MPs. González-Gil et al. (2018) evaluated the role of methanogenesis on the biotransformation of 20 MPs. This study showed that this process influenced for more than 50% of MPs biotransformation during AD. Within the MPs, some compounds such antibiotics (sulfamethoxazole, roxithromycin) and neuro-drugs (fluoxetine, citalopram) can be removed efficiently during AD. However, other compounds (fragrances and anti-inflammatories) are persistent and refractory to AD process (González-Gil et al. 2016). In addition to studying operational and microbiological parameters that influence the biotransformation of MPs during AD, the application of complementary advanced technologies (oxidation, microfiltration and ultrafiltration) may be consider without forgetting the use of bioassays for evaluating the potential toxicity of sludges (Liu et al. 2013).

Table 4 Removal efficiencies of different micropollutants during anaerobic digestion for environmental mitigation.

Micropollutant	Concentration in Sewage Sludge	Technology of Treatment	Removal Efficiencies (%)	Biotransformation Products	Toxicity Analyses	Ref.
β-Estradiol	0.6-1.2 µg/L[a]	Lab-scale anaerobic digesters operated in mesophilic (37°C) and thermophilic (55°C) conditions	<0%	-	-Estrogenic activity using Human Breast cancer cell line MCF-7 -Genetoxic activity using Ames[b] and Comet[c] tests	[1]
Galaxolide	141 µg/L[a]		8.2%	-		
Triclosan	38.1 µg/L[a]		17.1%	-		
Ibuprofen	11.8-25.6 µg/L[a]		28.5%	-		
Sulfamethoxazole	17.8 µg/L[a]		85%	-		
PAHs	1.7-3.6 µg/g[d]	Lab-scale anaerobic digesters operated in thermophilic conditions and with different pretreatment (thermal hydrolysis and ultrasound)	33-73%	-	Ecotoxicological tests with *Arthrobacter globiformis* and *Eisenia fetida*	[2]
PCBs	0.01-0.04 µg/g[d]		70%	-		
Diclofenac	995 µg/g[d]	Full-scale UASB reactor	99.7-99.8	-	Bioluminescence inhibition of *Vibrio fischeri*	[3];[4]
Carbamazepine	925.4 µg/g[d]		87.8-88.1	acridone-N-carbaldehyde		
Metoprolol	973.2µg/g[d]		94.2-95.1	-		
Triclosan	296µg/g[d]		92.9-96.6	methyltriclosan		

Notes: a Total concentration of micropollutants (solid and liquid phase); b point mutation in TA98 strain of *Salmonella typhimurium*; c performed in human leukocytes; d concentration determined in solid phase, UASB: upflow anaerobic sludge bed. **References:** [1] González-Gil et al. (2016); [2] Braguglia et al. (2015); [3] Butkovskyi et al. (2016); [4] Farré et al. (2008).

4. Perspectives of biosolids from anaerobic land disposal under the concept of circular economy (CE)

The CE consists of an economic system focused on maximizing the reuse of resources and products and minimizing their depreciation. In December 2015, the European Community introduced an action under the framework of CE called “Closing the loop” (European Commission 2015). This plan action defines two material cycles. The technical cycle relies in the use of mineral resources as production inputs, where products and their parts are designed and marketed in a way that they can be maintained and reused, maximizing their quality and their economic value. Within the biological cycle, resources used as production inputs have a biological origin, allowing for products to be safely discarded into the natural system once they reach their end of useful life. The system is meant to be both ecologically and economically restorative (Breure et al. 2018).

Figure 4 shows a schematic diagram of AD process under the strategy of CE. The wastewater treatment plants (WTP) using AD technology offer a suitable scenario for the application of the EC principle. The production of biogas and biosolids could be at the center of this sustainable system, in which the human waste supplies the production inputs. This energy can then go back to the community and WTP effluents and the biosolids can be used as a biofertilizer in crop production, so the cycle can go on and “close the loop” (Blades et al. 2017).

The land application of biosolids is a suitable option for recovery of mineral and organic constituents of soil for agricultural system. In fact, during the AD process, the biosolids has sizable concentrations of ammonium and orthophosphate, which are easily available for plant growth, allowing substitution or at least reduction of the use of chemical fertilizers (Da Ros et al. 2018). Despite these benefits, the biosolids present concentration of MPs such as PPCPs, metallic trace elements, and PAHs, which have a potential impact on the environment and human health. Because of their persistence, some MPs could be toxic and bioaccumulate with potential significant impacts including short-term and long-term toxicity, endocrine disrupting effect and antibiotic resistance.

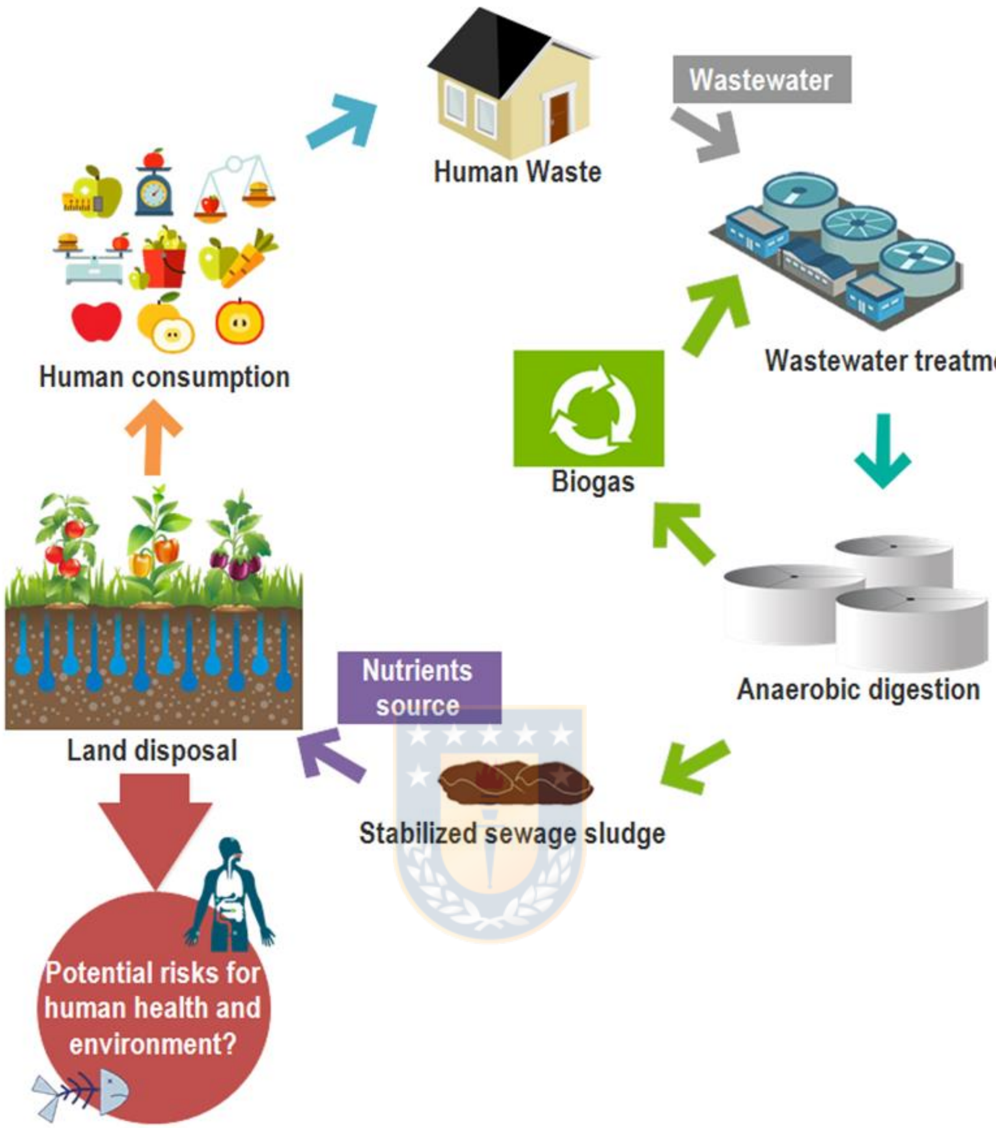


Figure 4. Schematic diagram of anaerobic digestion process under the strategy of circular economy.

This bioaccumulation is associated with the high lipid solubility property of a compound and its ability to accumulate in the fatty tissues of living organisms for a long time. These persistent compounds move up the food chain and they increase in concentration as they are processed and metabolized in certain tissues of organisms, increasing their toxicity in the environment (Grandclément et al. 2017). During the AD process, the transformation of MPs may occur depending on the physicochemical characteristics of the substance and conditions of the treatment. During this process, MPs can be removed or partially transformed to metabolites or in some instances left unchanged. In some cases, these transformation products can be more toxic and persistent than the initial compounds (Ebele et al. 2017).

The intrinsic complexity of human, agricultural and industrial residues from modern societies makes it impossible to identify, quantify and monitor all possible MPs present in a given sludge sample. Therefore, the establishment of permissible concentrations for an always limited number of MPs is not enough to define safe levels of chemical pollutants in a regulatory framework. An integrated measurement of the toxicity of a given sample can be performed with bioassays, which study specific biological changes (or endpoints) that occur in an organism after being exposed to a given substance or environmental sample. Bioassays can analyze the cumulative toxicity not only of the parental MPs, but also of their biotransformation products. For this reason, they can be used to evaluate the eco-toxicological effects of stabilized sewage sludge in both the receiving ecosystems and the human populations consuming the products from the amended soils (Gonzalez-Gil et al. 2016, see Figure 5). Bioassay endpoints can be on the molecular level (e.g., antibiotic resistance genes), microorganism level (e.g., the Microtox assay) or organism level (e.g., *Daphnia magna* or phytotoxicity assays) (Alvarenga et al. 2016). At the global level, life cycle analyses (LCA) are methodologies that allow the estimation of the environmental impacts of products, processes and services, potentially including all steps from raw material extraction to waste disposal (Cartes et al. 2018).

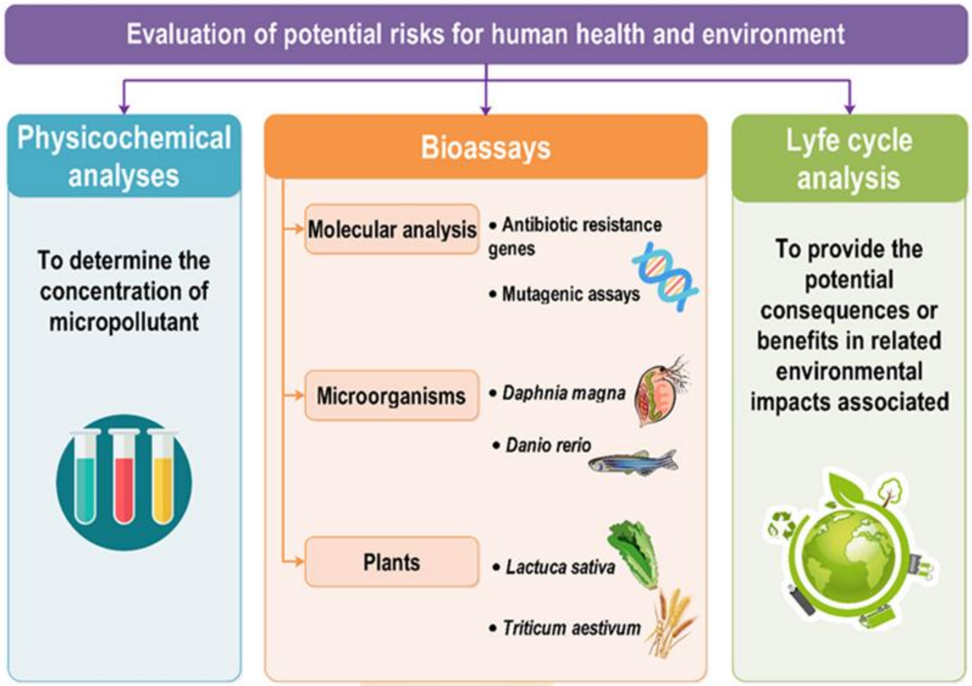


Figure 5. Schematic diagram of different analyses for evaluating the potential impacts and risks of the land disposal of biosolids.

5. Conclusions

Under the concept of CE, AD is an attractive technology that allowed to "close the loop" because this system is focused on maximizing the reuse of resources and minimizing their depreciation with the reuse of biogas and the land disposal of biosolids. Despite benefits for soil properties, this sewage sludge contains different MP such PPCPs, metallic trace elements and PAHs, which are associated to negative effects for the human health and the environment. These compounds are found in biosolids from AD with concentrations that depend of the physicochemical properties of MPs and the operational characteristics of the AD treatment (SRT, OLR). Because of their persistency, toxicity and bioaccumulative capacity, it is necessary to evaluate the potential risks of their presence on the biosolids. To date, there is no discharge guidelines and standards for most of these compounds. However, the EU water framework directive announces a list of 45 priority substances or groups of substance which include metals, pesticides, PAHs and endocrine disruptors. While is generally recognized that no chemical procedure will ever be sensitive enough to detect and quantify the thousands of potentially harmful compounds present in sewage, no guideline contemplates yet the use of bioassays in the evaluation of potential risks. For these reasons, it is imperative the study in detail of the effect of MPs using bioassays such phytotoxicity tests, mutagenic assays and antibiotic resistance genes.

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References

Abdullahi YA, Akunna JC, White NA, Hallett PD, Wheatley R (2008) Investigating the effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of municipal solid waste as soil amendment. Bioresource Technology 99(18):8631-8636. doi: 10.1016/j.biortech.2008.04.027.

Aguilera SM, Borie G, Peirano P, Rodriguez M, Grez I, Zunino H (2007) Chemical Characterization of sewage sludges in Chile and their potential utilization as amendment to reclaim soils for forestation purposes. Journal Plant Nutrition and Soil Science 30:1993-2003. doi: 10.1080/01904160701700400.

Alcock RE, Jones KC (1996) Dioxins in the Environment: A Review of Trend Data. Environment Science Technology 30, 3133-3143. doi: 10.1021/es960306z.

Alvarenga P, Mourinha C, Farto M, Palma P, Sengo J, Morais MC et al. (2016) Ecotoxicological assessment of the potential impact on soil pore water, surface and groundwater from the use of organic wastes as soil amendments. Ecotoxicology Environment Safety 126:102-110. doi: 10.1016/j.ecoenv.2015.12.019.

Appels L, Baeyens J, Degreve J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science 34:755–781. doi: 10.1016/j.pecs.2008.06.002.

Bester K (2003) Triclosan in a sewage treatment process-balances and monitoring data. Water Research 37(16):3891-3896. doi: 10.1016/S0043-1354(03)00335-X.

Blades L, Morgan K, Douglas R, Glover S, De Rosa M, Cromie T. et al. (2017). Circular biogas-based economy in a rural agricultural setting. Energy Proceedings 123:89-96. doi: 10.1016/j.egypro.2017.07.255.

Braga AFM, Zaiat M, Silva GHR, Fermoso FG (2017) Metal fractionation in sludge from sewage UASB treatment. Journal of Environmental Management 193: 98-107. doi: 10.1016/j.jenvman.2017.01.070.

Braguglia CM, Coors A, Gallipoli A, Gianico A, Guillon E, Kunkel U, et al. (2015). Quality assessment of digested sludges produced by advanced stabilization processes. Environmental Science and Pollution Research 22(10):7216-7235. doi: 10.1007/s11356-014-3090-6.

Breure, A.M., Lijzen, J.P.A., Maring, L. (2018). Soil and land management in a circular economy. Science of the Total Environment 624, 1125-1130. doi: 10.1016/j.scitotenv.2017.12.137.

Butkovskyi, A., Ni, G., Leal, L.H., Rijnaarts, H.H.M., Zeeman, G. (2016). Mitigation of micropollutants for black water application in agriculture via composting of anaerobic sludge. Journal Hazardous Materials 303, 41-47. doi: 10.1016/j.jhazmat.2015.10.016.

Cartes J, Neumann P, Hospido A, Vidal G (2018) Life cycle assessment of management alternatives for sludge from sewage treatment plants in Chile: does advanced anaerobic digestion improve environmental performance compared to current practices? Journal of Material Cycles and Waste Management 1-11. doi: 10.1007/s10163-018-0714-9.

Céspedes R, Petrovic M, Raldúa D, Saura U, Piña B, Lacorte S, et al (2004). Integrated protocol for the determination of endocrine disrupting activity in the surface waters and sediments using biological techniques by recombinant yeast assay and chemical analysis by LC-ESI-MS. Analytical Bioanalytical Chemistry 378:687-708. doi: 10.1007/s00216-003-2303-5.

Chamorro S, Monsálvez E, Hernández V, Becerra J, Mondaca MA, Piña B, et al (2010) Detection of Estrogenic Activity from Kraft Mill Effluents by Yeast Estrogen Screen. Bulletin Environmental Contamination and Toxicology 84:165-169. doi: 10.1007/s00128-009-9917-z.

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresource Technology 99(10):4044–4064. doi:10.1016/j.biortech.2007.01.057.

Chu S, Metcalfe CD (2007) Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. Journal Chromatography A. 1164: 212-218. doi: 10.1016/j.chroma.2007.07.024.

Clara M, Gans O, Windhofer G, Krenn U, Hartl W, Braun K, et al (2011) Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. Chemosphere 82:1116-1123. doi: 10.1016/j.chemosphere.2010.11.041.

CWTS (2019) Software VOS viewer Visualizing Scientific Landscapes. Version 1.6.10, Centre for Science and Technology Studies, Leiden University. http://www.vosviewer.com/.

Da Ros C, Libralato G, Ghirardini AV, Radaelli M, Cavinato C (2018) Assessing the potential phytotoxicity of digestate from winery wastes. Ecotoxicology Environmental Safety 150:26-33. doi: 10.1016/j.ecoenv.2017.12.029.

De Jesus Gaffney V, Almeida CMM, Rodrigues A, Ferreira E, Benoliel MJ, Cardoso VV (2015) Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. Water Research 72 :199-208. doi: 10.1016/j.watres.2014.10.027.

De Souza Pereira M, and Kuch B (2005) Heavy metals, PCDD/F and PCB in sewage sludge samples from two wastewater treatment facilities in Rio de Janeiro State, Brazil. Chemosphere 60: 844-853. doi: 10.1016/j.chemosphere.2005.01.079.

Ebele AJ, Abdallah MAE, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminations 3(1):1-16. doi: 10.1016/j.emcon.2016.12.004.

European Commission (2015) Closing the Loop—an EU Action Plan for the Circular Economy COM (2015) 614.

Farré M, Asperger D, Kantiani L, González S, Petrovic, M, Barceló D (2008) Assessment of the acute toxicity of triclosan and methyl triclosan in wastewater based on the bioluminescence inhibition of Vibrio fischeri. Analytical Bioanalytical Chemistry 390(8):1999-2007. doi: 10.1007/s00216-007-1779-9.

Gao P, Ding Y, Li H, Xagoraraki I (2012) Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. Chemosphere 88:17-24. doi: 10.1016/j.chemosphere.2012.02.017.

Garrido T, Mendoza J, Arriagada F (2012) Changes in the sorption, desorption, distribution, and availability of copper, induced by application of sewage sludge on Chilean soils contaminated by mine tailings. Journal Environment Science 24:912-918.

Göbel A, Thomsen A, McArdell CS, Alder AC, Giger W, Theiß N, et al (2005) Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. J. Chromatography A 1085(2):179-189. doi: 10.1016/j.chroma.2005.05.051.

Golet EM, Strehler A, Alder AC, Giger W (2002) Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. Analytical Chemistry 74(21):5455-5462. doi: 10.1021/ac025762m.

Gondek K, Mierzwa-Hersztek M, Kopeć M (2018) Mobility of heavy metals in sandy soil after application of composts produced from maize straw, sewage sludge and biochar. Jouranl Environment Management 210:87-95. doi: 10.1016/j.jenvman.2018.01.023.

Gonzalez-Gil L, Mauricio-Iglesias M, Serrano D, Lema JM, Carballa M (2018) Role of methanogenesis on the biotransformation of organic micropollutants during anaerobic digestion. Science Total Environment 622:459-466. doi: 10.1016/j.scitotenv.2017.12.004.

Gonzalez-Gil L, Papa M, Feretti D, Ceretti E, Mazzoleni G, Steimberg N, et al (2016) Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge?. Water Research 102: 211-220. doi: 10.1016/j.watres.2016.06.025.

Grandclément C, Seyssiecq I, Piram A, Wong-Wah-Chung P, Vanot G, Tiliacos N et al (2017). From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. Water Research 111: 297-317. doi: 10.1016/j.watres.2017.01.005.

Heidler J, Halden RU (2009) Fate of organohalogens in US wastewater treatment plants and estimated chemical releases to soils nationwide from biosolids recycling. Journal Environment Monitory. 11:2207-2215. doi: 10.1039/b914324f.

Jia A, Wan Y, Xiao Y, Hu J (2012) Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. Water Research 46:387-394. doi: 10.1016/j.watres.2011.10.055.

Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Research 41(5): 1013-1021. doi: 10.1016/j.watres.2006.06.034.

Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL, Cahill JD, et al (2006) Survey of organic wastewater contaminants in biosolids destined for land application. Environment Science and Technology 40(23): 7207-7215. doi: 10.1021/es0603406.

Kupper T, Berset JD, Etter-Holzer R, Furrer R, Tarradellas J (2004) Concentrations and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 54:1111-1120. doi: 10.1016/j.chemosphere.2003.09.023.

Lai KM, Scrimshaw MD, Lester JN (2002) The effects of natural and synthetic steroid estrogens in relation to their environmental occurrence. Critical Review Toxicology 32(2): 113-132. doi: 10.1080/20024091064192.

Leiva C, Ahumada I, Sepúlveda B, Richter P (2010) Polychlorinated biphenyl behavior in soils amended with biosolids. Chemosphere 79: 273-277. doi: 10.1016/j.chemosphere.2010.01.043.

Lindberg RH, Fick J, Tysklind M (2010) Screening of antimycotics in Swedish sewage treatment plants – Waters and sludge. Water Research 44: 649-657. doi: 10.1016/j.watres.2009.10.034.

Liu JL, Wong MH (2013) Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environment International 9: 208-224. doi: 10.1016/j.envint.2013.06.012.

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J et al (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environment 473: 619-641. doi: 10.1016/j.scitotenv.2013.12.065.

Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Brock Biology of Microorganisms. San Francisco: Benjamin Cummings.

Malinauskaite J, Jouhara H, Czajczyńska D, Stanchev P, Katsou E, Rostkowski P et al. (2017) Municipal solid waste management and waste-to-energy in the context of a circular economy and energy recycling in Europe. Energy 141: 2013-2044. doi: 10.1016/j.energy.2017.11.128.

Maria ICD, Chiba MK, Costa A, Berton RS (2010) Sewage sludge application to agricultural land as soil physical conditioner. Revista Brasileira de Ciência do Solo 34(3): 967-974. doi: 10.1590/S0100-06832010000300038.

Martín J, Camacho-Muñoz MD, Santos JL, Aparicio I, Alonso E (2012a) Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. Journal Environment Management 102: 18-25. doi: 10.1016/j.jenvman.2012.02.020.

Martín J, Camacho-Muñoz D, Santos JL, Aparicio I, Alonso E (2012b) Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. Jouranl Hazardous Material 239-240:40-47. doi: 10.1016/j.jhazmat.2012.04.068.

Martín J, Santos JL, Aparicio I, Alonso E (2015) Pharmaceutically active compounds in sludge stabilization treatments: Anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Science of the Total Environment 503: 97-104. doi: 10.1016/j.scitotenv.2014.05.089.

Mattana S, Petrovicová B, Landi L, Gelsomino A, Cortés P, Ortiz O, et al (2014) Sewage sludge processing determines its impact on soil microbial community structure and function. Applied Soil Ecological 75:150–161. doi: 10.1016/j.apsoil.2013.11.007.

Mesquita SR, Van Drooge B, Barata C, Vieira N, Guimarães L, Piña B (2014) Toxicity of atmospheric particle-bound PAHs: an environmental perspective. Environment Science Pollution Research 21: 11623–11633. doi: 10.1007/s11356-014-2628-y.

Muñoz M, Garrido MA, Gómez-Rico MF, Font R (2018) PCDD/F determination in sewage sludge composting. Influence of aeration and the presence of PCP. Science Total Environment 616: 763-773. doi: 10.1016/j.scitotenv.2017.10.249.

Neumann P, Barriga F, Álvarez C, González Z, Vidal G (2018) Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound – thermal (55°C) pre-treatment. Bioresorce Technology 262: 42-51. doi: 10.1016/j.biortech.2018.03.057.

Neumann P, González Z, Vidal G (2017) Sequential ultrasound and low temperature thermal pretreatment: process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. Bioresource Technology 234: 178-187. doi: 10.1016/j.biortech.2017.03.029.

Neumann P, Pesante S, Venegas M, Vidal G (2016) Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Review Environment Science Biotechnoly 15(2): 173-211. doi: 10.1007/s11157-016-9396-8.

Paraíba LC, Queiroz SCN, Maia A de HN, Ferracini VL (2010) Bioconcentration factor estimates of polycyclic aromatic hydrocarbons in grains of corn plants cultivated in soils treated with sewage sludge. Science of the Total Environment 408: 3270-3276. doi: 10.1016/j.scitotenv.2010.04.026.

Peysson W, Vulliet E (2013) Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography–time-of-flight-mass spectrometry. Journal Chromatography A 1290: 46-61. doi: 10.1016/j.chroma.2013.03.057.

Piña B, Bayona JM, Christou A, Fatta-Kassinos D, Guillon E, Lambropoulou D et al (2018) On the contribution of reclaimed wastewater irrigation to the potential exposure of humans to antibiotics, antibiotic resistant bacteria and antibiotic resistance genes – NEREUS COST Action ES1403 position paper. Journal Environmental Chemistry Engineering doi: 10.1016/j.jece.2018.01.011.

Reyes-Contreras C, Matamoros V, Ruiz I, Soto M, Bayona JM (2011) Evaluation of PPCPs removal in a combined anaerobic digester-constructed wetland pilot plant treating urban wastewater. Chemosphere 84:1200-1207. doi: 10.1016/j.chemosphere.2011.06.003.

Rogers HR (1996) Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. Science of the Total Environment 185(1-3): 3-26. doi: 10.1016/0048-9697(96)05039-5.

Rosinska A, Karwowska B (2017) Dynamics of changes in coplanar and indicator PCB in sewage sludge during mesophilic methane digestion. Journal Hazardous Material 323: 341-349. doi: 10.1016/j.jhazmat.2016.04.016.

Silva Oliveira A, Bocio A, Beltramini T, Magosso A, Domingo J, Segura-Muñoz S (2007) Heavy metals in untreated/treated urban effluent and sludge from a biological wastewater treatment plant. Environment Science Pollution Research International 14:483-489. doi: doi.org/10.1065/espr2006.10.355.

Stasinakis AS (2012) Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour. Technol. 121:3432-440. doi: 10.1016/j.biortech.2012.06.074.

Stasinakis AS, Gatidou G, Mamais D, Thomaidis NS, Lekkas TD (2008) Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. Water Research 42:1796-1804. doi: 10.1016/j.watres.2007.11.003.

Stevens JL, Northcott GL, Stern GA, Tomy GT, Jones KC (2003) PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in UK sewage sludge: survey results and implications. Environment Science Technology 37(3): 462-467. doi: 10.1021/es020161y.

Tampio E, Salo T, Rintala J (2016) Agronomic characteristics of five different urban waste digestates. Journal Environment Management 169: 293-302. doi: 10.1016/j.jenvman.2016.01.001.

Thanh P.M. Ketheesan B. Yan Z. Stuckey D (2016) Trace metal speciation and bioavailability in anaerobic digestion: A review. Biotechnology Advances 34(2): 122-136. doi: 10.1016/j.biotechadv.2015.12.006.

United States Environmental Protection Agency (USEPA). (2002). Supplemental guidance for developing soil screening levels for superfund sites. Washington (D.C): Office of Emergency and Remedial Response.

Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environment Toxicology Pharmacology 13 (2): 57–149. http://dx.doi.org/10.1016/S1382-6689(02)00126-6.

Vignais PM, Colbeau A (2004) Molecular biology of microbial hydrogenases. Current Issues Molecular Biology 6(2): 159-188.

Villar L, Garcia O (2006) Effect of anaerobic digestion and initial pH on metal bioleaching from sewage sludge. Journal Environment Science Health Part 41: 211-222. doi: 10.1080/10934520500354516.

Wolejko E, Wydro U, Jabłońska-Trypuć A, Butarewicz A, Loboda T (2018) The effect of sewage sludge fertilization on the concentration of PAHs in urban soils. Environment Pollution 232: 347-357. doi: 10.1016/j.envpol.2017.08.120.

World Health Organization (WHO) (2006) Guidelines for the Safe Use of Wastewater, Excreta and Greywater: Policy and Regulatory Aspects. World Health Organization.

Yang L, Jin M, Tong C, Xie S (2013) Study of dynamic sorption and desorption of polycyclic aromatic hydrocarbons in silty-clay soil. Journal Hazardous Material 244-245: 77-85. doi:10.1016/j.jhazmat.2012.11.029.

Zhang Q, Hu J, Lee DJ (2016) Biogas from anaerobic digestion processes: Research updates. Renewable Energy 98:108-119. doi: 10.1016/j.renene.2016.02.029.

Zhang XX, Zhang T, Fang HH (2009) Antibiotic resistance genes in water environment. Apply Microbiology Biotechnology 82(3): 397-414. doi: 10.1007/s00253-008-1829-z.

Zhou H, Wu C, Onwudili JA, Meng A, Zhang Y, Williams PT (2015) Polycyclic aromatic hydrocarbons (PAH) formation from the pyrolysis of different municipal solid waste fractions. Waste Management 36: 136-146. doi: 10.1016/j.wasman.2014.09.014.

CAPÍTULO VI

INFLUENCE OF ANAEROBIC DIGESTION WITH PRETREATMENT ON THE PHYTOTOXICITY OF SEWAGE SLUDGE

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Influence of anaerobic digestion with pretreatment on the phytotoxicity of sewage sludge

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Abstract

The aim of this study is to evaluate the influence of sewage biosolids phytotoxicity by anaerobic digestion with pretreatment. The phytotoxicity was evaluated on sewage sludge (SS) and biosolids that came from conventional anaerobic digestion (CAD) and anaerobic digestion with a pretreatment by sequential ultrasound and low-thermal hydrolysis, called advance anaerobic digestion (AAD). To compare the phytotoxicity, eight elutriate concentrations (0.5%-100% v/v) from SS, CAD and AAD were studied on three testing plants: *Lactuca sativa*, *Raphanus sativus* and *Triticum aestivum*. The percentage of seed germination inhibition, root elongation and germination index (GI) were evaluated. GI is a phytotoxicity indicator that combines seed germination and root growth, therefore reflecting a more complete estimation of toxicity. Phytotoxicity assays showed that SS, CAD and AAD elutriates have a beneficial effect on *R. sativus*. Similar results were observed for *T. aestivum* for CAD and AAD, with GI values up to 80% in both biosolids. Only for SS, moderate toxicity was observed in *T. aestivum*. Moreover, *L. sativa* showed GI values below 50% for SS and CAD, which reflected high toxicity. Only for AAD, no presence of phytotoxic substances was observed in *L. sativa*. This study concluded that biosolids from AAD improved the plants' development with a GI above 78% with respect to biosolids from SS and CAD and reduced the phytotoxicity of sewage biosolid.

1. Introduction

In recent decades, sewage sludge (SS) generation has increased due to the aerated technology (i.e., activated sludge) used in sewage treatment (Stiborova et al. 2017). The accumulation of non-stabilized SS generates environmental problems for populations and ecosystems (Alvarenga et al. 2016). Conventional anaerobic digestion (CAD) generates a stabilized SS (called biosolid) that can be used as a soil amendment with beneficial effects (Fuentes et al. 2004). In terms of methane production, CAD can be improved by using a pretreatment (Neumann et al. 2016). Thus, advanced anaerobic digestion (AAD) consisting of a sequential ultrasound and low-thermal hydrolysis (55 °C-90 °C) has been proposed as an economically feasible pretreatment technology (Carvajal et al. 2013; Dhar et al. 2012; Neumann et al. 2017).

The potential risks of biosolids being used as a soil amendment are studied by ecotoxicological assessment (Walter et al. 2006). Specifically, the phytotoxicity assay is a quick tool to evaluate the ecotoxicity of biosolids due to its simplicity and sensitivity (Gerber et al. 2017). A common phytotoxicity indicator is the germination index (GI). The GI is index forming a cumulative toxicity evaluation of sewage sludges and biosolids elutriates taking into consideration both seed germination and root growth (Oleszczuk 2009; Rodriguez-Rodriguez et al. 2011), where GI below 50% indicates high toxicity effect (Roig et al. 2012). Many researchers have studied the phytotoxicity effects of biosolids from CAD (Fuentes et al. 2004; Ramírez et al. 2008; Walter et al. 2006) and showed that the percentages of relative seed germination of *Lepidium sativus* (cress) and *Hordeum vulgare* (barley) were more than 70% (Fuentes et al. 2004). However, the GI revealed that this biosolid had toxic effects less than 50% on *L. sativus* seeds. Similar results have been reported for the germination of *L. sativus*, *H. vulgare* and *Avena sterilis*, which show an inhibition GI of below 60% for biosolids from CAD (Walter et al. 2006). By contrast, Ramírez et al. (2008), show an 80% inhibitory effect of composted biosolids on *Brassica rapa* (turnip), *Lolium perenne* (ray grass) and *Trifolium pratense* (red clover). However, studies of the effect of pretreatment on the phytotoxicity of biosolids have not yet been reported.

For this reason, the aim of this study was to evaluate the influence of anaerobic digestion with pretreatment on the phytotoxicity of sewage sludge-derived elutriates.

2. Materials and methods

2.1. Biosolid and elutriate samples

The sewage sludge (SS) samples were collected from a municipal wastewater treatment plant in Concepción, Biobío Region, Chile (36° 48’ S, 73° 08’ W). The plant treats wastewater of approximately 500,000 inhabitants. Two types of biosolids were used. One biosolid came from conventional anaerobic digestion (CAD), and the other came from advanced anaerobic digestion (AAD). The pretreatment in AAD consists of a sequential process; ultrasound followed by a low-temperature thermal process according to Neumann et al. (2017). All samples were stored at 4 °C for 20 days until use.

Elutriate samples were separately obtained from SS and biosolids from CAD and AAD according to Pandard et al. (2006) and Wilke et al. (2008). The elutriates were obtained by adding distilled water to the samples at a ratio of 1:10 m/v (wet weigh). The mix was agitated at room temperature for 24 h. Then, it was centrifuged at 3000 rpm (2000 x g) in a Sigma 3-16 L centrifuge (Sigma Laborzentrifugen GmbH, Germany) for 10 min. Finally, the supernatant was filtered through a membrane filter of pore size 0.45 µm (Whatman). The elutriates were stored at 4°C for 7 days until use.

2.2. Physicochemical characterization

Physicochemical parameters, pH, and conductivity were measured with OAKTON portrait multiparameter (PC650-480485). The total phosphorus (TP), nitrogen of the ammonium (NH_4^+-N) and total nitrogen (TN) of the elutriates were determined with specific determination kits, line Spectroquant-Nova 60 of Merck®. For the sludge samples, organic matter, as the chemical oxygen demand (COD), total organic carbon (TOC) (catalytic combustion by oxidation method and NDIR detection), volatile solids (VS) and total solids (TS) and the following parameters were determined according to Standard Methods (APHA 2012). The TP was determined by the vanadomolybdophosphoric acid colorimetric method, while the NH_4^+-N and Total Kjeldahl Nitrogen (TKN) were determined by the titrimetric method. Arsenic (As) and selenium (Se) were determined by hydride generation/atomic absorption spectrometry; cadmium (Cd), copper (Cu), nickel (Ni), lead

(Pb), and zinc (Zn) were determined by flame atomic absorption spectrometry (direct air-acetylene flame method); and mercury (Hg) was determined by cold-vapor atomic absorption spectrometry.

2.3. Phytotoxicity assay

The phytotoxicity assay was conducted according to 208 OECD/OCDE (2006) methodology. Two dicotyledonous plants, *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish), and one monocotyledonous plant, *Triticum aestivum* (wheat), were used. This assay considers the indirect phytotoxicity, which consists of a representative extract from the whole matrix of SS, CAD and AAD. Eight elutriates of different concentrations in %v/v, denoted by E, were evaluated: 0.5%E, 1%E, 5%E, 10%E, 20%E, 40%E, 80%E and 100%E. Distilled water was used as a control (C).

Ten seeds were placed in Petri dishes, and 5 mL of the appropriate elutriate concentration was added. The seeds were incubated at room temperature (25 °C-27 °C) under continuous light conditions. The incubation time depend on the plant species with 6 dayS for *L. sativa*, 5 days for *R. sativus*, and 5 days for *T. aestivum*. The bioassay was conducted in triplicate. The toxicity endpoints that were assessed, were seed germination, root elongation and sprout length (Fuentes et al. 2004). The percentage of inhibition was calculated for seed germination and root elongation, as follows:

$$Inhibitionpercentage = \frac{C - E}{C} * 100\%$$ **Equation 1**

where *C* corresponds to the value (number of seeds germinated or root length) in the control and *E* corresponds to the value (number of seeds germinated or root length) in the elutriate (Oleszczuk and Hollert 2011).

The germination index (GI) was determined using equation 2. The GI was toxicologically classified according to Roig et al. (2012) as follows: beneficial effect (GI ≥ 100), no presence of phytotoxic substances (100 >GI ≥ 80), moderate toxicity (80 > GI >50), and high toxicity (50 ≥ GI).

$$GI = \frac{S_E}{S_C} * \frac{RL_E}{RL_C} * 100\%$$ **Equation 2**

where S_E and S_C are the number of germinated seeds on the elutriate and the control and RL_E and RL_C are the mean root length of the seeds on the elutriate and the control, respectively (Tiquia et al. 1996).

2.4. Data analysis

Statistical differences between treatments were assessed through a non-parametric analysis of variance ($p<0.05$, Kruskal Wallis test). InfoStat (version 2016) and the Origin 8 software were used to perform the statistical analysis.

3. Results and discussion

3.1. Physicochemical characterization of biosolids and elutriates from SS, CAD and AAD

Table 1 shows the physicochemical characterization and heavy metal content of SS and the biosolids from CAD and AAD. In all samples, the pH was slightly alkaline with values between 7.7 and 8.8. These results were consistent with those reported in the literature, which fluctuated from 7.9 to 8.7 for similar treatments (Fuentes et al. 2004; Ramírez et al. 2008; Walter et al. 2006). In the case of conductivity, the values ranged from 1.7 to 2.0 mS/cm with non-significant differences between SS, CAD and AAD (p>0.05). For the COD, VS and TS, the biosolids from CAD and AAD showed concentrations and percentages that were 2 times lower than those obtained for SS. This difference is expected because CAD and AAD reduce the solid content of SS through the hydrolysis of high-molecular-weight compounds (Neumann et al. 2016). Regarding the nutrient content, the TKN contents were 33% and 30% higher in the biosolids from CAD and AAD than they were in SS, respectively. However, non-significant differences between SS and biosolids (CAD and AAD) were observed in NH_4^+-N content, with values that ranged from 12.9 to 15.6 g/kg. In anaerobic digestion, the the main forms of nitrogen are organic nitrogen and NH_4^+-N, due to the absence of an adequate electron acceptor in anaerobic digestion that hinders the oxidation of nitrogen, therefore NO_x- is unusual in anaerobic digestion effluent (Yang et al. 2018). Hence, nitrogen in the biosolids were mainly in the organic form representing more than 70% of the TKN content. This result is similar to those found by Da Ros et al. (2018), who reported organic nitrogen content of 63%-76% for biosolids from CAD.

Moreover, for SS and biosolids from CAD and AAD, the Hg, Ni and Pb contents were below 3 mg/kg without significant differences ($p>0.05$). In contrast, the Cu and Zn contents increased by 63% and 79%, respectively, with the stabilization of SS. According to Chipasa (2003), CAD can cause an increase of 50%-99% in the content of heavy metal (Zn, Pb, Cu and Cd) relative to SS, which is related to the degradation of organic and inorganic matter. Carballa et al. (2009), related the average solid removal in CAD and AAD with the heavy metal increase of around 50%. According to the above, the low amounts of Hg, Ni and Pb in the SS make the reduction of organic matter in biosolids does not influence an increase in its concentration. Besides, the behavior of Cu could also be affected by precipitation and accumulation as inorganic salts in the reactor (Neumann et al. 2018). Despite these values, the heavy metal content in SS and the biosolids from CAD and AAD were below European (Directive 86/278/EEC) and Chilean (DS 04) legislation, (Council of the European Union 1986; Gobierno de Chile 2010), which indicate that it can be used for soil amendment.

Table 1. Physicochemical characterization and heavy metal content of biosolids from conventional anaerobic digestion (CAD), advanced anaerobic digestion (AAD) and sewage sludge (SS).

Parameter	**SS**	**CAD**	**AAD**
Conductivity (mS/cm)	1.720±0.001	1.740±0.001	2.050±0.001
COD (g/L)	118.62±8.07	68.59±8.96	62.25±4.45
pH	7.77±0.01	8.76±0.01	8.43±0.01
TS (%)	10.43±0.65	6.31±0.12	5.30±0.03
VS (%)	7.08±0.33	4.24±0.08	3.51±0.01
TKN (g/kg)	45.15	68.01	64.80
NH_4^+-N (g/kg)	12.90	13.98	15.62
TP (g/kg)	1.08	1.40	1.28
Cu (mg/kg)	167	288	455
Hg (mg/kg)	1.33	1.28	1.94
Ni (mg/kg)	<1	<1	<1
Pb (mg/kg)	<3	<3	<3
Zn (mg/kg)	147	656	702

Data expressed as mean ± standard deviation. SS: sewage sludge; CAD: conventional anaerobic digestion; AAD: advanced anaerobic digestion; COD: chemical oxygen demand; TS: total solids; VS: volatile solids; TKN: Total Kjeldahl Nitrogen; TP: total phosphorus.

By contrast, Table 2 shows physicochemical characterization of the SS, CAD and SS elutriates. Similar behaviors to those of the solid matrix (Table 1) were observed for COD and TOC concentrations in elutriates from SS, CAD and AAD. In this case, the values were 1.7 times lower in the CAD and AAD elutriates than in SS. Similarly, the NH_4^+-N concentrations ranged between 91.0 and 101.5 mg/L in the elutriates, and it represented the predominant nitrogen form with 91%-98% of TN. The heavy metal content was not determined in the SS, CAD and AAD elutriates because it was expected they are present as metal precipitates in the sludge flocs of the solid matrix, and therefore, their values would be negligible (Chipasa 2003). This is supported by Alvarenga et al. (2016), who observed that the Cd, Cr, Cu, Ni and Zn contents were below the detection limit in elutriates from biosolids from CAD. For this reason, it was unlikely the presence of heavy metal would influence the phytotoxicity of the elutriates.

Table 2. Physicochemical characterization of elutriate from sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) biosolids.

Parameter	SS	CAD	AAD
Conductivity (mS/cm)	0.860±0.024	0.920±0.017	0.800±0.003
COD (g/L)	0.30±0.06	0.18±0.02	0.18±0.002
pH	7.59±0.28	7.05±0.13	7.63±0.19
TN (mg/L)	111.0±1.2	93.0±1.2	94.0±1.2
NH_4^+-N (mg/L)	101.5±9.7	91.3±2.2	91.0±5.0
TP (mg/L)	42.30±0.13	42.30±0.13	34.20±0.13
TOC (mg/L)	42.58±0.01	20.25±0.01	24.82±0.01

Data expressed as mean ± standard deviation. SS: sewage sludge; CAD: conventional anaerobic digestion; AAD: advanced anaerobic digestion; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus; TOC: total organic carbon.

3.2. Phytotoxicity effects of SS, CAD and AAD on plant growth development

Figure 1 shows the relative root length of *R. sativus*, *T. aestivum* and *L. sativa* with different elutriate percentages of SS, CAD and AAD. For R. sativus, SS, CAD and ADD had positive effects on the root elongation, with values that were 24%-44%, 15%-29% and 12%-36% higher in the different elutriates than in the control, respectively. In the case of *T. aestivum*, in SS, CAD and AAD, the mean root lengths of different elutriate percentages were above 5.61 cm, 2.62 cm and 3.80 cm, respectively, which corresponded to the values of the control samples. Similar to *R. sativus*, the root elongation was stimulated by SS, CAD and AAD. Likewise, for *L. sativa*, the mean root lengths of the elutriate percentages were similar to the control samples, with values close to 0.38 cm, 0.71 cm and 0.43 cm in SS, CAD and AAD, respectively. By contrast, Figure 2 shows the relative sprout length of *R. sativus*, *T. aestivum* and *L. sativa* with different elutriate percentages of SS, CAD and AAD. For *R. sativus* and *T. aestivum*, similar behaviors were observed. In SS, CAD and AAD, the relative sprout lengths were similar to the values of the control samples, which were in the range of 1.11-2.06 cm, 0.89-2.06 cm and 0.73-2.11 cm, respectively. In these cases, the sprout elongation was stimulated, whereas differences close to 10% between the elutriate percentages and the control were observed ($p>0.05$). For L. sativa, the mean sprout length showed a decrease that was close to 50% and 64% in SS and CAD, respectively, in relation to the control samples. However, the mean sprout lengths were relative constant, with values close to 0.30 cm in all the elutriate percentages for AAD.

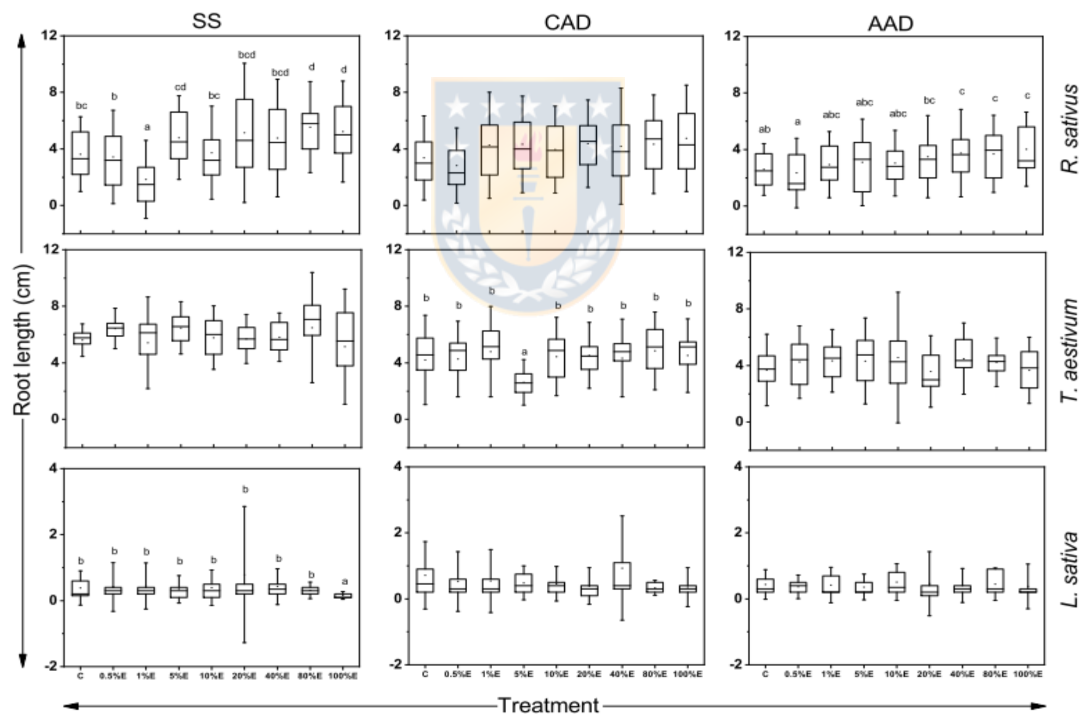

Figure 1. Relative root length (cm) of plant tested (*R. sativus*, *T. aestivum*, and *L. sativa*) with different elutriate percentages (C:0%, 0.5%E, 1%E, 5%E, 10%E, 20%E, 40%E, 80%E, and 100%E) of sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD). Treatments marked with the same letter were not significantly different (Kruskal-Wallis *p>0.05*).

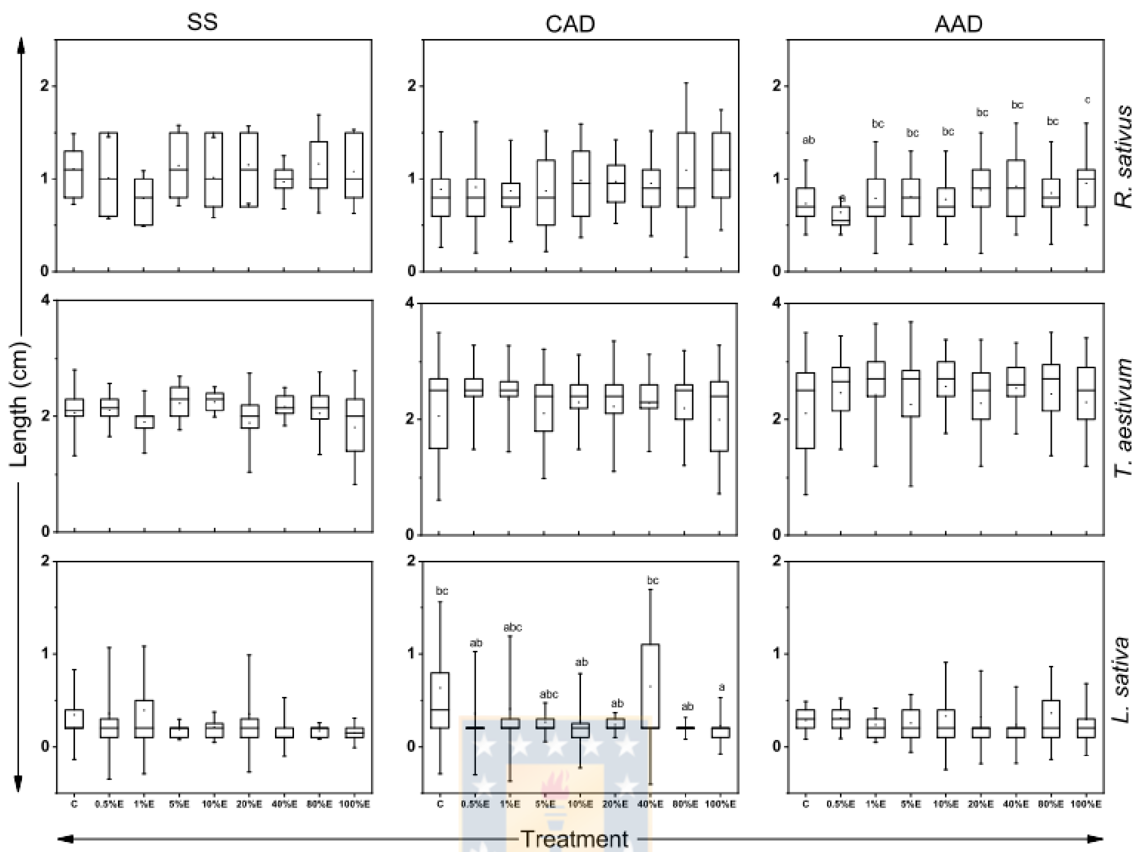

Figure 2. Relative sprout length (cm) of plant tested (*R. sativus*, *T. aestivum*, and *L. sativa*) with different elutriate percentages (C:0%, 0.5%E, 1%E, 5%E, 10%E, 20%E, 40%E, 80%E, and 100%E) of sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD). The treatments, marked with the same letter, were not significantly different (Kruskal-Wallis $p>0.05$).

Consequently, the same tendencies of the relative root and sprout length (Figures 1 and 2) were observed in the inhibition percentages. Figure 3 shows the relative seed and root inhibition of different elutriate percentages from SS, CAD and AAD. In this case, a positive value indicates an adverse effect on the plant development, and a negative value indicates a beneficial effect. The biosolids-derived elutriates had an effect on the seed germination of the tested plants (Fig. 3), for *R. sativus* and *T. aestivum* had a positive effect on the seed germination, with percentages of -30%-4% and -11%-15% in the biosolids samples (CAD and AAD), respectively. For *L. sativa*, the germination inhibition shows an adverse effect in all samples (SS, CAD and AAD), with values of

18%-24% (Fig. 3). In this case, *L. sativa* appeared to be more sensitive than *R. sativus* and *T. aestivum*. Moreover, the results from root inhibition showed the same behavior for *L. sativa*. The elutriate percentages of SS, CAD and AAD caused negative effects with values that varied from -107% to 58%. However, for *R. sativus* and *T. aestivum*, the root inhibition showed beneficial results for the three elutriates, with -53%-10%, -42%-38% and -54%-10% for SS, CAD, and AAD, respectively. These results were in agreement with the literature, where seeds of root crops, cereals and legumes such *R. sativus* and *T. aestivum* contain more food reserve than seeds of leafy plants; thus, they tend to have lower sensitivity to toxicity (Tiquia et al. 1996). Similarly, Alvarenga et al. (2016) reported that for seed germination, *L. sativa* was more sensitive than *L. perenne* to different SS and compost elutriates. The same tendencies were observed in Bank and Schultz (2005), who tested *L. sativa*, *Panicum miliaceum* (*P. miliaceum*), *R. sativum*, *Trifolium pretense* (*T. pretense*) and *T. aestivum* for sensitivity to petroleum contamination in germination tests. One possible explanation for this behavior was the presence of some chemical pollutant such NH_4^+-N, which can inhibit plant development (Fuentes et al. 2006). The toxicity of this compound was often expressed as root inhibition characterized by damage in the root, where the production of short, thick, less branched and darkly colored roots is the specific feature of the toxicity (Li et al. 2013). For *L. sativa* exposed to SS-derivated elutriates effects on the thickness of the root and darkening at the end of the root were evidenced. Furthermore, Tabatabaei et al. (2006) established that the optimum level of NH_4^+-N concentration for lettuce was 36 mg/L. In this study, for SS, CAD and AAD, the NH_4^+-N concentrations were above 91.0 mg/L (Table 2).

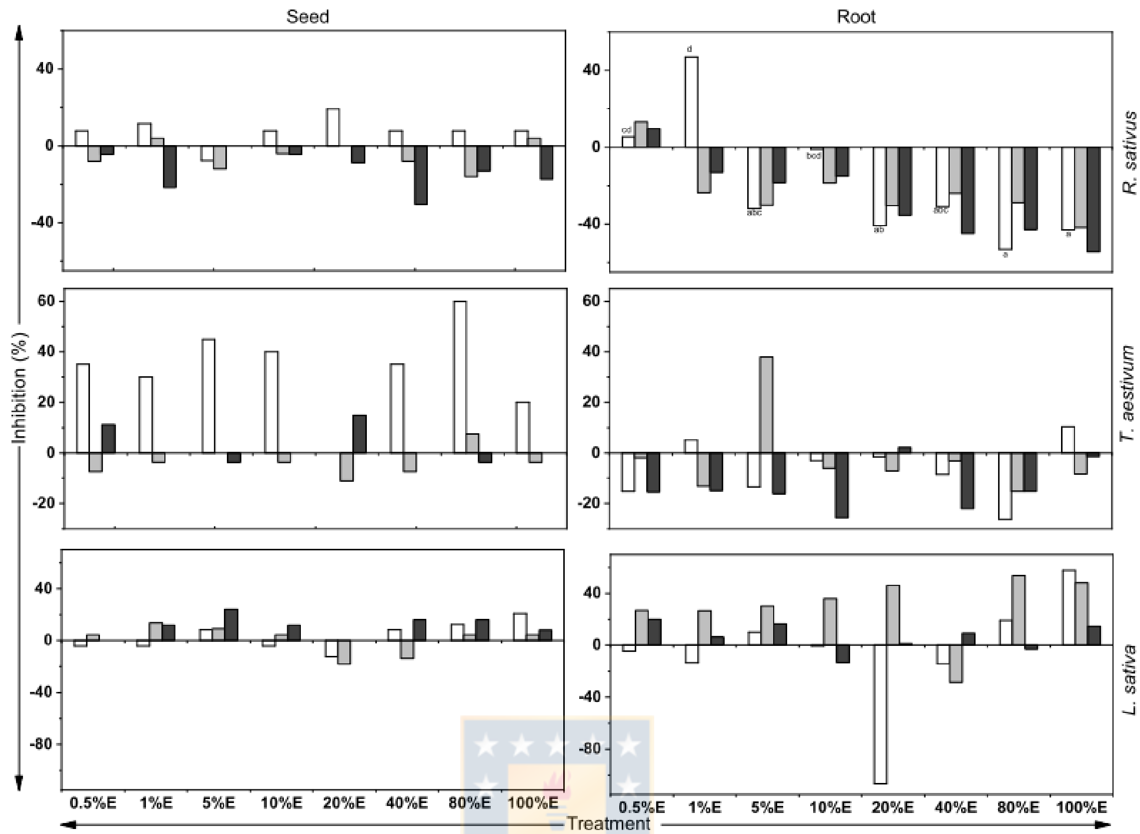

Figure 3. The relative seed and root inhibition (%) of the plant tested (*R. sativus*, *T. aestivum* and *L. sativa*) with different elutriate percentages (0.5%E, 1%E, 5%E, 10%E, 20%E, 40%E, 80%E, and 100%E) of sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD). Columns, within each type of sample, marked with the same letter were not significantly different (Kruskal-Wallis $p>0.05$). Elutriates from □ sewage sludge, ■ conventional anaerobic digestion, ■ advanced anaerobic digestion.

Equally important, the inhibition of *R. sativus*, *T. aestivum* and *L. sativa* seed germination was 3.5 times more sensitive than that of root elongation. Divided opinions about the sensitivity of the root length and seed germination can be observed in the literature. Oleszczuk (2008) reported that the root inhibition of *L. sativum* was more sensitive than seed germination, with values ranging from-20% to 75% and -30% to -10%, respectively. However, Oleszczuk and Hollert (2011) showed that seed germination inhibition was slightly more sensitive than root length inhibition. In this case, the percentage fluctuated in the ranges of 0%-90% and 95%-100% for the seed germination and root length, respectively.

Moreover, SS elutriates had the most severe inhibition effect in all tested plants (*L. sativa*, *R. sativus* and *T. aestivum*), with percentages rising to almost 60% of the inhibition (Figure 3). This result could be related to the low stability stage of the SS, and it is corroborated with the GI percentages. Figure 4 shows the GI of the plants tested for SS, CAD and AAD elutriates. For *R. sativus*, the GI reported values above 80%, which indicated no presence of phytotoxic substances in SS, CAD and AAD (Figure 4a). In the case of *T. aestivum*, biosolids-derived elutriates (CAD and AAD) have positive effects on GI, with percentages above 109% and 103%, respectively (Figure 4b). Meanwhile, SS-derived elutriates produced moderate and high toxicity in *T. aestivum* with GI below 76% for 0.5%E, 40%E and 100%E and a GI of 46% for 80%E. As seen in Figure 3, *L. sativa* was the most sensitive plant tested (Figure 4c), with GI fluctuated in the range 33%-231%, 45%-148%, and 78%-105% for SS, CAD, and AAD, respectively.

In general, for the different endpoints evaluated, the source of the sample (SS, CAD or AAD-derived elutriated) showed a greater influence on the phytotoxicity than the concentrations of elutriated applied. That's why in Table 3 summarizes the toxicity classification of SS, CAD and AAD for 100%E, according to GI. Considering the three plants that were evaluated, AAD elutriates showed the most beneficial effects. The results from the Kruskal-Wallis test (analysis of variance non-parametric) while considering the GI from E8 evidenced significant differences ($p<0.05$) between the samples studied (SS, CAD, AAD). It is reported that the degree of stability of the organic matter is a critical factor in biosolids phytotoxicity (Fuentes et al. 2006). Fuentes et al. (2006) compared the phytotoxicity of different types of sludge on *H. vulgare* and *L. sativum*. In this case, the GI values were 35% higher in the sludge that was stabilized with anaerobic digestion (GI: 75%) than non-stabilized (GI: 48%) for *H. vulgare*. This behavior can be explained by the partial degradation of organic pollutants, which reduces the organic matter and modifies its distribution and availability (Petersen et al. 2003; Smith and Tibbett 2004). As mentioned in Tables 1 and 2, the organic matter concentration, measured as COD, was reduced 40%-42% in CAD and AAD. Furthermore, Ramirez et al. (2008) indicated that the phytotoxicity of SS was reduced when post-treatments such as composting and thermal-drying were applied. In this study, GI values over 80% showed that the phytotoxicity of biosolids was lessened when a pretreatment (sequential ultrasound and low-thermal hydrolysis) was added (Table 3). The pretreating in the AAD process promotes both the hydrolysis mechanism and the solubilization of the SS (Neumann et al. 2018) that promote the biosolids stabilization and contributes to the decrease of phytotoxicity thereof (Fuentes et al. 2006).

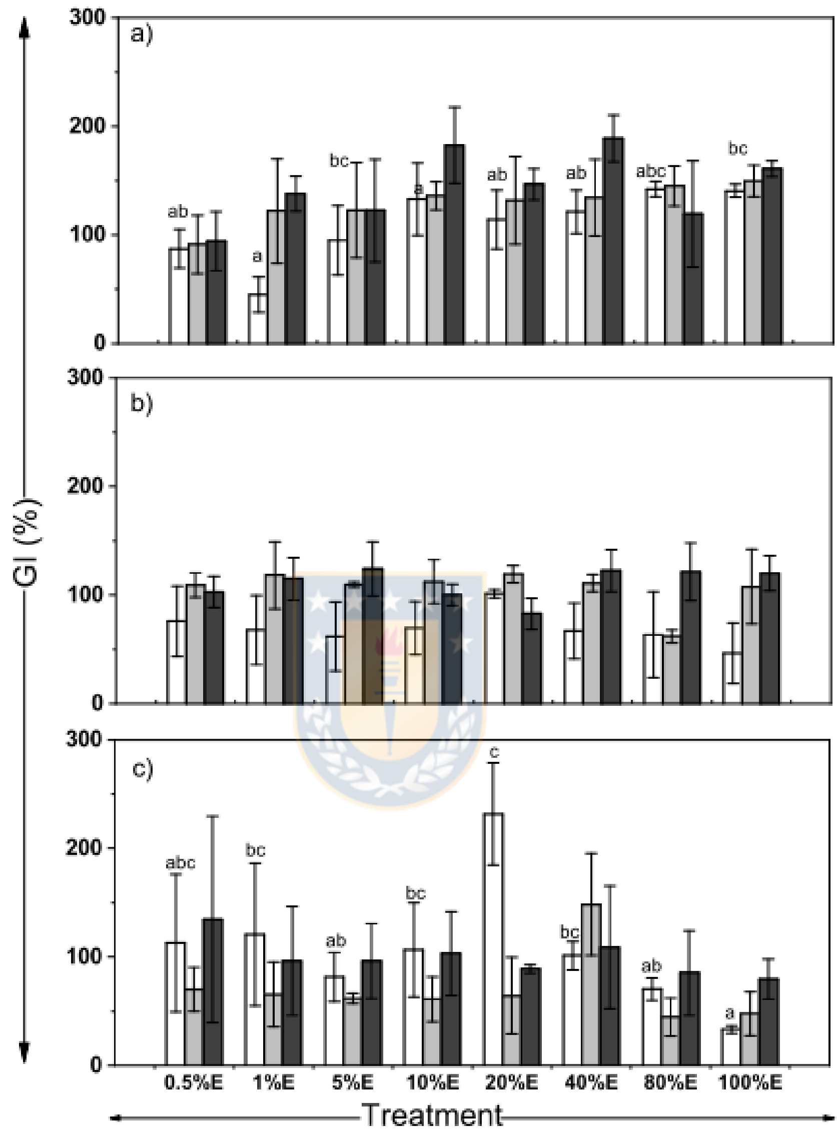

Figure 4. Germination Index (%) relative to plant tested (a) *R. sativus*, b) *T. aestivum* and c) *L. sativa*) with different elutriate percentages (0.5%E, 1%E, 5%E, 10%E, 20%E, 40%E, 80%E, and 100%E) from sewage sludge (SS) and biosolids (BS). (mean ± S.D., n=3) Columns, within each type of sample, marked with the same letter, were not significantly different (Kruskal-Wallis *p>0.05*). Elutriates from □ sewage sludge, ■ conventional anaerobic digestion, ■ advanced anaerobic digestion.

Table 3. Toxicity classification according to Germination Index of elutriate (100%E) from sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) biosolids.

Sample	*R. sativus*	*T. aestivum*	*L. sativa*
SS	Beneficial effect	Moderate toxicity	High toxicity
CAD	Beneficial effect	Beneficial effect	High toxicity
AAD	Beneficial effect	No presence of phytotoxic substances	No presence of phytotoxic substances

SS: sewage sludge; CAD: conventional anaerobic digestion; AAD: advanced anaerobic digestion. GI was toxicologically classified according to Roig et al. (2012) in *beneficial effect* (GI ≥ 100), *no presence of phytotoxic substances* (100 > GI ≥ 80), *moderate toxicity* (80 > GI > 50), and *high toxicity* (50 ≥ GI).

Phytotoxicity assay can be direct when the whole matrix is used or indirect when a representative extract is used (Ramírez et al. 2008). It is known that direct phytotoxicity avoids extraction problem and represent more closely the real exposure conditions, but the high content of organic matter in the SS and biosolids could hide the effect of some pollutants (Alvarenga et al. 2016; Ramírez et al. 2008). Within this scenario, elutriate or water-extracts are a suitable matrix to evaluate phytotoxicity, because elutriate contains an important bioavailable fraction of pollutants (Alvarenga et al. 2016; Ramírez et al. 2008). Considering the aforementioned, this study is presented as a first step in the evaluation of AAD biosolids for soil application. Showing promising results with a decreased in phytotoxicity of the AAD-derivated elutriated. Currently, a second stage of the AAD-biosolids evaluation is being carried out using a mixture of reference soil and the biosolids.

4. Conclusions

In this study, *L. sativa* was the most sensitive plant tested, with percentages of germination and root length inhibition in the range of -107% to 58%, -29% to 55%, and -13% to 24% in SS, CAD and AAD. With regard to the effect of different samples on GI, SS showed moderate and high toxicity for *T. aestivum* and *L. sativa*. For CAD in *R. sativus* and *T. aestivum*, GI indicated a positive effect on the plant development. However, different behaviors were observed for *L. sativa*, with GI values below 50%, which showed high toxicity. Moreover, for AAD, beneficial effects or no presence of phytotoxic substances were reported for tested plants. The GI results of up to 90% showed that the application of a pretreatment (sequential ultrasound and low-thermal hydrolysis) enhanced the phytotoxicity of the sewage biosolids.

Acknowledgments

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References

Alvarenga, P., Mourinha, C., Farto, M., Palma, P., Sengo, J., Morais, M. C., et al (2016). Ecotoxicological assessment of the potential impact on soil porewater, surface and groundwater from the use of organic wastes as soil amendments. Ecotoxicology and Environmental Safety, 126, 102–110, https://doi.org/10.1016/j.ecoenv.2015.12.019

APHA, American Public Health Association, (2012). Standard methods for examination of water and wastewater (22nd ed.). Washington: American Public Health Association.

Banks, M. K., & Schultz, K. E. (2005). Comparison of Plants for Germination Toxicity Tests in Petroleum-Contaminated Soils. Water, Air, and Soil Pollution, 167(1–4), 211–219. http://doi.org/10.1007/s11270-005-8553-4

Carballa, M., Omil, F., & Lema, J. M. (2009). Influence of different pretreatments on anaerobically digested sludge characteristics: Suitability for final disposal. Water, Air, and Soil Pollution, 199(1–4), 311–321. http://doi.org/10.1007/s11270-008-9880-z

Carvajal, A., Peña, M., & Pérez-Elvira, S. (2013). Autohydrolysis pretreatment of secondary sludge for anaerobic digestion. Biochemical Engineering Journal, 75, 21–31. https://doi.org/10.1016/j.bej.2013.03.002

Chipasa, K. B. (2003). Accumulation and fate of selected heavy metals in a biological wastewater treatment system. Waste Management, 23(2), 135–143, https://doi.org/10.1016/S0956-053X(02)00065-X

Council of the European Union. Directive 86/278/EEC on the protection of the environment, and in particular of the soil when sewage sludge is used in agriculture (1986). European Union: Official Journal of the European Communities.

Da Ros, C., Libralato, G., Ghirardini, A. V., Radaelli, M., & Cavinato, C. (2018). Assessing the potential phytotoxicity of digestate from winery wastes. Ecotoxicology and Environmental Safety, 150, 26–33, https://doi.org/10.1016/j.ecoenv.2017.12.029

Dhar, B. R., Nakhla, G., & Ray, M. B. (2012). Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. Waste Management, 32(3), 542–549, https://doi.org/10.1016/j.wasman.2011.10.007

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M. I., Ortuño, J. F., & Meseguer, V. F. (2004). Phytotoxicity and heavy metals speciation of stabilised sewage sludges. Journal of Hazardous Materials, 108(3), 161–169, https://doi.org/10.1016/j.jhazmat.2004.02.014

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M. I., Pérez-Marín, A. B., Ortuño, J. F., et al. (2006). Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. Environmental Pollution, 143(2), 355–360, https://doi.org/10.1016/j.envpol.2005.11.035

Gerber, M. D., Lucia, T., Correa, L., Neto, J. E. P., & Correa, É. K. (2017). Phytotoxicity of effluents from swine slaughterhouses using lettuce and cucumber seeds as bioindicators. Science of The Total Environment, 592, 86–90, https://doi.org/10.1016/j.scitotenv.2017.03.075

Gobierno de Chile. 2010. Reglamento Para El Manejo de Lodos Generados En Plantas de Tratamiento de Aguas Servidas. Chile: Biblioteca del Congreso Nacional de Chile: Ley Chile. http://www.leychile.cl/Navegar/?idNorma=1007456&idVersion=2010-04-26&idParte.

Li, S. X., Wang, Z. H., & Stewart, B. A. (2013). Responses of crop plants to ammonium and nitrate N. InAcademic Press (Ed.), Advances in Agronomy (pp. 205-397) Amsterdam: Elsevier.

Neumann, P., Barriga, F., Álvarez, C., González, Z., & Vidal, G., (2018). Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound-thermal (55°C) pre-treatment. Bioresource Technology, 262, 42–51. https://doi.org/10.1016/j.biortech.2018.03.057.

Neumann, P., González, Z., & Vidal, G. (2017). Sequential ultrasound and low-temperature thermal pretreatment: Process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. Bioresource Technology, 234, 178–187, https://doi.org/10.1016/j.biortech.2017.03.029

Neumann, P., Pesante, S., Venegas, M., & Vidal, G. (2016). Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Reviews in Environmental Science and Bio/Technology, 15(2), 173–211, https://doi.org/10.1007/s11157-016-9396-8

OECD/OCDE. (2006). Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. OECD Publishing, https://doi.org/10.1787/9789264070066-en

Oleszczuk, P. (2008). Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicology and Environmental Safety, 69(3), 496–505, https://doi.org/10.1016/j.ecoenv.2007.04.006

Oleszczuk, P. (2009). Testing of different plants to determine influence of physico-chemical properties and contaminants content on municipal sewage sludges phytotoxicity. Environmental Toxicology, NA-NA. http://doi.org/10.1002/tox.20470

Oleszczuk, P., & Hollert, H. (2011). Comparison of sewage sludge toxicity to plants and invertebrates in three different soils. Chemosphere, 83(4), 502–509, https://doi.org/10.1016/j.chemosphere.2010.12.061

Pandard, P., Devillers, J., Charissou, A. M., Poulsen, V., Jourdain, M. J., Férard, et al. (2006). Selecting a battery of bioassays for ecotoxicological characterization of wastes. Science of The Total Environment, 363(1–3), 114–125, https://doi.org/10.1016/j.scitotenv.2005.12.016

Petersen, S.O., Petersen, J., & Rubaek, G.H. (2003). Dynamics and plant uptake of nitrogen and phosphorus in soil amended with sewage sludge. Applied Soil Ecology,24(2), 187–195, https://doi.org/10.1016/S0929-1393(03)00087-8

Ramírez, W. A., Domene, X., Ortiz, O., & Alcañiz, J. M. (2008). Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresource Technology, 99(15), 7168–7175, https://doi.org/10.1016/j.biortech.2007.12.072

Rodriguez-Rodriguez, C. E., Jelić, A., Llorca, M., Farré, M., Caminal, G., Petrović, M., … Vicent, T. (2011). Solid-phase treatment with the fungus Trametes versicolor substantially reduces pharmaceutical concentrations and toxicity from sewage sludge. Bioresource Technology, 102(10), 5602–5608. http://doi.org/10.1016/j.biortech.2011.02.029

Roig, N., Sierra, J., Nadal, M., Martí, E., Navalón-Madrigal, P., Schuhmacher, M., & Domingo, J. L. (2012). Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants. Science of The Total Environment, 425, 99–109, https://doi.org/10.1016/j.scitotenv.2012.03.018

Smith, M.T.E., & Tibbett, M. (2004). Nitrogen dynamics under *Lolium perenne* after a single application of three different sewage sludge types from the same treatment stream. Bioresource Technology, 91(3), 233-241, https://doi.org/10.1016/S0960-8524(03)00205-0

Stiborova, H., Kolar, M., Vrkoslavova, J., Pulkrabova, J., Hajslova, J., Demnerova, K., & Uhlik, O. (2017). Linking toxicity profiles to pollutants in sludge and sediments. Journal of Hazardous Materials, 321, 672–680. https://doi.org/10.1016/j.jhazmat.2016.09.051

Tabatabaei, S. J., Fatemi, L. S., & Fallahi, E. (2006). Effect of ammonium: nitrate ratio on yield, calcium concentration, and photosynthesis rate in strawberry. Journal of Plant Nutrition, 29(7), 1273-1285, https://doi.org/10.1080/01904160600767575

Tiquia, S. M., Tam, N. F. Y., & Hodgkiss, I. J. (1996). Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environmental Pollution, 93(3), 249–256, https://doi.org/10.1016/S0269-7491(96)00052-8

Walter, I., Martínez, F., & Cala, V. (2006). Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses. Environmental Pollution, 139(3), 507–514, https://doi.org/10.1016/j.envpol.2005.05.020

Wilke, B.-M., Riepert, F., Koch, C., & Kühne, T. (2008). Ecotoxicological characterization of hazardous wastes. Ecotoxicology and Environmental Safety, 70(2), 283–293, https://doi.org/10.1016/j.ecoenv.2007.10.003

Yang, Y., Zhang, Y., Li, Y., Zhao, H., & Peng, H. (2018). Nitrogen removal during anaerobic digestion of wasted activated sludge under supplementing Fe (III) compounds. Chemical Engineering Journal, 332, 711–716. https://doi.org/10.1016/j.cej.2017.09.133

CAPÍTULO VII

PHYTOTOXICITY OF BIOSOLIDS FOR SOIL APPLICATION: INFLUENCE OF CONVENTIONAL AND ADVANCED ANAEROBIC DIGESTION WITH A SEQUENTIAL PRE-TREATMENT

Venegas M., Leiva A.M., Bay-Schmith E., Silva J. and Vidal G. (2019) Phytotoxicity of biosolids for soil application: Influence of different anaerobic digestion processes. *Environmental Technology and Innovation*. 16, 100445. DOI: 10.1016/j.eti.2019.100445

Phytotoxicity of biosolids for soil application: influence of conventional and advanced anaerobic digestion with a sequential pre-treatment

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Abstract

The aim of this study was to evaluate the influence of different anaerobic digestion processes on phytotoxicity of biosolids (BS) for soil application. Three different plants species, *Lactuca sativa*, *Raphanus sativus*, and *Triticum aestivum* were subjected to seven treatments of different BS (T: 5-1000 g/kg). In this case, the BS samples come from the stabilization of sewage sludge (SS) using separately conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) with a sequential pre-treatment of ultrasound followed by a low-temperature thermal process. To evaluate the phytotoxicity, germination inhibition percentages, the root and sprout growth, the germination index (GI) and EC_{50} were determined. The results showed that the application of SS with a concentration of 250 g/kg inhibited the germination of *L. sativa*, *R. sativus* and *T. aestivum* with percentages that varied between 50-100%. This study concludes that the application of BS of 100 g/kg shows beneficial effects (GI ≥100) or no presence of phytotoxicity (100 > GI ≥ 80). This result was corroborated by the EC_{50} average of 164 g/kg and 159 g/kg for all the endpoints and plants evaluated with BS of CAD and AAD, respectively.

1. Introduction

Under the scenario of climate change and the threat of availability of natural resources such as water and phosphorus, it is necessary a paradigmatic shift from “end-of-pipe-approach” to “resources-oriented-approach”. Moreover, under this new perspective, the life cycle of materials have to be considered in the framework of the circular economy (Alvarenga et al., 2017; Papa et al., 2017). In this context, the wastewater treatment plants (WWTPs) should tend to be designed with technologies that allow for closing cycles and recovery of resources (Papa et al., 2017).

Biosolids (BS) (stabilized sewage sludge (SS)) are resources generated by WWTPs which could be considered raw materials used to soil amendment in agriculture due to their physicochemical properties (Alvarenga et al., 2017). The use of BS on soil offers economic and environmental benefits because they: a) allow the recycling of micro- and macronutrients (nitrogen, phosphorus, and potassium); b) enhance soil organic carbon storage; c) promote the formation of stable aggregates; d) improve water holding capacity, soil cationic exchange and aeration and e) promote erosion resistance. (Alvarenga et al., 2017; Antilén et al., 2014). However, the use of BS as soil improvers faces important challenges of food and ecosystem security. An inefficient stabilization of SS, its mean with ratio of volatile solids/total solids (VS/TS) above 0.6 and with values of VS removal below 40% (Braguglia et al., 2015), can cause the soil contamination with heavy metals due to the extractability of metal are greater in less stabilized sludges (Fuentes et al., 2006), eutrophication of water bodies and risks associated with pathogenic microorganisms and micropollutants (Wu et al., 2015).

The stabilization of SS using conventional anaerobic digestion (CAD) reduces the solids contents, produces biogas (60-70% methane) and generates BS which have a potential use as a soil amendment (Tiwary et al., 2015). However, CAD is limited by the hydrolysis of organic solids in the SS. For improving the hydrolysis stage, different pre-treatment (physical, chemical and biological) have been extensively studied (Neumann et al., 2016). The use of pre-treatment in anaerobic digestion process is known as advanced anaerobic digestion (AAD). The implementation of AAD with sequential ultrasound and thermal pre-treatment at low temperature (55-90°C) can increase the methane yield between 19-30% (Carvajal et al., 2013; Neumann et al., 2018). This combination of ultrasound and thermal (at low temperature) treatments allows the physical disruption of the flocs and provide favorable conditions for the reactivation of the endogenous enzymatic activity (Neumann et al., 2017). Conversely, the effect of AAD on BS properties has not yet been fully reported.

Bioassays are interesting tools for evaluating the ecotoxicological effects of BS from CAD and AAD, and the phytotoxicity is a useful assay because it is a quick and simple tool for the evaluation of ecotoxicity (Gerber et al., 2017). Ramírez et al. (2008) evaluated the potential phytotoxicity effects (*Brassica rapa*, *Lolium perenne*, and *Trifolium pratense*) of BS on soil from different stabilization methods. In this study, a strong negative correlation (r≈0.9) between the degree of organic matter stability and toxicity was observed. Regarding EC_{50} of germination, for BS from composting with application doses between 60-670 g/kg, these values fluctuated between 478-610 g/kg, whereas the EC_{50} for fresh sludge with application doses between 12-228 g/kg varied between 17-36 g/kg. These results suggested that when the stability degree of sludge is higher, the toxicity effects are lower (Ramírez et al., 2008). Oleszczuk (2008) used *Lepidium sativum* to study the phytotoxicity of BS (SS stabilized by composting), for application doses of 6%, the percentages of germination inhibition varied between -20 a -10%, whereas for application doses of 24%, significant germination inhibitions were determined with percentages between 20 a 100%. Moreover, Adamcová et al. (2016) reported that BS from CAD have a strong toxic effect on *Sinapis alba* with growth inhibition percentages that fluctuated from 70 to 100% for application doses of 10-100%.

Few studies have considered the phytotoxicity of BS from CAD (Adamcová et al., 2016; Domene et al., 2011; Kinney et al., 2012; Ramírez et al., 2008) and the study of the phytotoxicity of BS from AAD is even more scarce. For that reason, the aim of this study was to evaluate the influence of different anaerobic digestion processes on BS phytotoxicity for soil application.

2. Materials and methods

2.1. Biosolids samples and artificial soil

The BS used came from two lab scale anaerobic reactor. One reactor was operated under conventional conditions (CAD) and the other under advanced conditions (AAD) used a sequential pre-treatment (Figure 1). The pre-treatment consists in a ultrasound step (at 26 kHz and with a specific energy of 2000 kJ/kg total solids) follows by the low-temperature thermal process (55°C) according to Neumann et al. (2017). The sewage sludge (SS) used to feed the anaerobic reactor were from a municipal wastewater treatment plant in Concepción, Biobío Region, Chile (36° 48' S, 73° 08' W). A composite sample was used. A daily sample was taken during the operational time of the reactor. All the samples were stored at 4°C until use. The composite sample was centrifugated and dried before to be used in the assay. To evaluate the phytotoxicity on soil, artificial soil was used as a control. Their composition was according to OCDE guidelines 207 (OECD 1984) with 10% of peat, 20% of kaolin clay and 70% of sand.

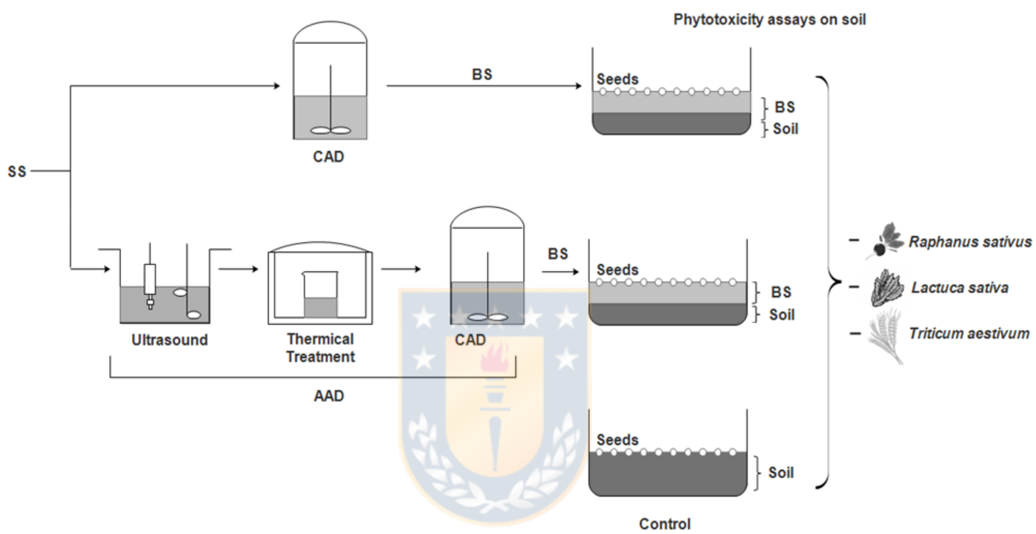

Figure 1. Experimental schematic diagram design used for phytotoxicity assays in the soil. Where SS: sewage sludge, CAD: conventional anaerobic digestion, AAD: advanced anaerobic digestion, BS: biosolids.

2.2. Physicochemical characterization

Physicochemical characterization of SS, BS (CAD and AAD), and artificial soil were realized. chemical oxygen demand (COD), volatile solids (VS), and total solids (TS) were measured according to Standard Methods for the Examination of Water and Wastewater (APHA 2012). Total phosphorus (TP), potassium (K), the nitrogen of the ammonium (NH_4^+-N) and total nitrogen (TN) were determined with specific determination kits, line Spectroquant-Nova 60 of Merck®. Conductivity and pH were measured with a portrait multiparameter OAKTON (PC650-480485).

2.3. Phytotoxicity assay

Considering the plant used in different phytotoxicity assay (Kinney et al., 2012; Ramírez et al., 2008) and the recommended by the methodology used (OECD/OCDE, 2006). As testing plants one monocotyledonous and two dicotyledonous plants were used, *Triticum aestivum*, *Lactuca sativa*, and *Raphanus sativus*, respectively. Seven treatments (T, the term "treatment" will continue to be used as a reference to different experiments carried out)); T1: 5 g/kg, T2: 50 g/kg, T3: 100 g/kg, T4: 250 g/kg, T5: 500 g/kg, T6: 750 g/kg, and T7: 1000 g/kg, were used. The artificial soil was used as a control (C).

Following 208 OECD (2006) methodology, ten seeds were placed in Petri dishes and 45 g of the corresponding treatment was added. Continuous light and room temperature (24-26°C) were the incubation conditions. The bioassay was carried in triplicate. The endpoints assessed were seed germination, root elongation and, sprout length (Fuentes et al., 2004). Inhibition of seed germination and root elongation, as the germination index (GI, equation 1) were calculated as mentioned by Venegas et al. (2018). The GI was classified into beneficial effect (GI ≥ 100), no presence of phytotoxic substances (100 > GI ≥ 80), moderate toxicity (80 > GI >50), and high toxicity (50 ≥ GI) (Roig et al., 2012).

$$GI = \frac{S_T}{S_C} * \frac{RL_T}{RL_C} * 100$$

Equation 1

where S_T and S_C are the number of germinated seeds on the treatment and the control and RL_T and RL_C are the mean root length of the seeds on the treatment and the control, respectively.

2.4. Data analysis

Shapiro-Wilk test was applied to verify the normality of the data set. Then, non-parametric analysis of variance was used to evaluated the stadistical differences between treatment ($p<0.05$, Kruskal Wallis test). The statistical analysis, including EC_{50} value, was performed with the software InfoStat (version 2016) and Origin 8.

3. Results and discussion

3.1. Physicochemical characterization of SS and BS from CAD and AAD

Table 1 shows the physicochemical characterization of SS, BS from CAD and AAD and soil used as a control (C). For C and SS, the pH values were slightly acid with averages of 6.07 and 6.80, respectively. In the case of BS from CAD and AAD, these values showed a neutral tendency with averages of 7.23 and 7.43, respectively. Similar results were reported for stabilized BS with anaerobic digestion present in literature with pH ranges that varied between 6.50-7.54 (Alvarenga et al., 2008; Carbonell et al., 2009; Ferreiro-Domínguez et al., 2011). Nutrients concentrations in C were related to the composition of peat and kaolin presented in the mix. The main minerals of kaolin were kaolinite ($Al_2Si_2O_5(OH)_4$) and muscovite ($KAl_2(AlSi_3O_{10})(OH)_2$) (Arias and Sen, 2009). For this reason, the nutrient contribution of soil was reflected in the K content which was between 1.79 to 3.12 times higher in C than in SS, CAD, and AAD. In the case of BS, the K content remained within the reported values between 1.3-2.9 g/kg (Alvarenga et al., 2008; Moreira et al., 2008; Ramírez et al., 2008). Moreover, the average concentrations of TP that varied between 19.3-34.9 g/kg were similar to those reported for stabilized BS with anaerobic digestion (Ferreiro-Domínguez et al., 2011; Ramírez et al., 2008). The TP in AAD was approximately 55% higher than the content in SS and CAD ($p<0.05$). This behavior could be related to the effect of pre-treatment on cell lysis which increases the availability of some compounds, phosphorus between them (Reyes-Contreras et al., 2018). Previous studies report that CAD led to increase of almost all elements monitored up to 378% higher in BS compared to SS, and pre-treatment led to increases of up to 58% in the concentration of the studied elements in the BS compared to CAD (Appels et al., 2010; Neumann et al., 2018).

Regarding the TN content, non-significant differences between BS (CAD and AAD) were reported ($p>0.05$) with variations between 62.1-65.3 g/kg. The SS and BS (CAD and AAD) used in this study were study before considering metal and microcontaminant concentration (Reyes-Contreras et al., 2018; Venegas et al., 2018). The metal content in the SS and the biosolids (CAD and AAD) were bellow European (Directive 86/278/EEC) and Chilean (DS 04) legislation, which indicate that it can be used for soil amendment (Venegas et al., 2018). Future investigation related with the ecotoxicity of BS from AAD, should related other compounds, as microcontaminant, present in the SS and the effect of the stabilization technology. Reyes-Contreras et al. (2018), studied the influence of the pre-treatment over the removal of some microcontaminant, suggesting that pre-treatment could improve the removal of sequestered or highly hydrophobic compounds through their solubilization and increased bioavailability during anaerobic digestion. However, an ecotoxicological approach is needed to evaluate the suitability of the BS as an amendment to the soil.

Table 1. Physicochemical characterization of sewage sludge and biosolids from conventional and advanced anaerobic digestion biosolids at different concentration ratio.

Parameter	Units	C	SS	CAD	AAD
VS/TS	-	0.07	0.7	0.6	0.5
COD	g/L	28.65±5.47	172.31±16.11	174.95±55.17	150.25±64.28
pH	-	6.07±0.21	6.80±0.06	7.23±0.07	7.43±0.13
TP	g/kg	10.7±0.5	19.3±2.5	18.9±0.7	34.9±1.6
K	g/kg	4.75±0.07	1.52±0.07	1.89±0.07	2.65±0.07
TN	g/kg	19.76.0±5.6	78.42±5.2	62.130±0.5	65.31±14.3
NH_4^+-N	g/kg	4.7±1.2	36.9±0.48	17.5±0.48	22.6±0.48

C: control soil; SS: sewage sludge; CAD: Conventional Anaerobic Digestion; AAD: Advanced Anaerobic Digestion; VS: volatile solids; TS: total solids; COD: chemical oxygen demand; TP: total phosphorous; K: potassium; TN: total nitrogen; NH4+-N: nitrogen of the ammonium.

3.2. Effects of SS, CAD, and AAD on plants' growth

Figure 2 shows the relative root length of *R. sativus*, *T. aestivum* and *L. sativa* for different application doses (treatments) of SS, CAD, and AAD. These bioassays showed negative effects of SS samples on the three plants tested. For *R. sativus* and *L. sativa*, the root elongation decreased between 32-98% and 33-68%, respectively for all application doses of SS. The same behavior was observed in the root length of *T. aestivum* for application doses between 50-250 g/kg which showed decreases between 15-71%. However, non-significant differences with respect to the control plant were observed for the application dose of 5 g/kg corresponding to SS ($p>0.05$). Regarding the effects of CAD and AAD on the root length of *R. sativus*, the results were similar. In the case of the application dose of 5 g/kg, the root length increased between 7-8% with respect to the control plant. In contrast, reductions of the root growth between 17-51% were determined for application doses of 50-250 g/kg and these reductions were greater to 90% when the doses were above 500 g/kg. For *T. aestivum*, the application dose of 50 g/kg of CAD caused an increase in the root growth of 77% whereas the application dose of 5 g/kg of AAD increased this parameter on 61%. In the case of the behavior of *L. sativa*, application doses between 5-100 g/kg of CAD had non-significant differences with the control species (*p>0.05*). For application doses of 50 g/kg corresponding to AAD, the root growth increased on 40%. In general, samples from CAD and AAD have positive effects on the root length of the three plants species evaluated for application doses below 50 g/kg.

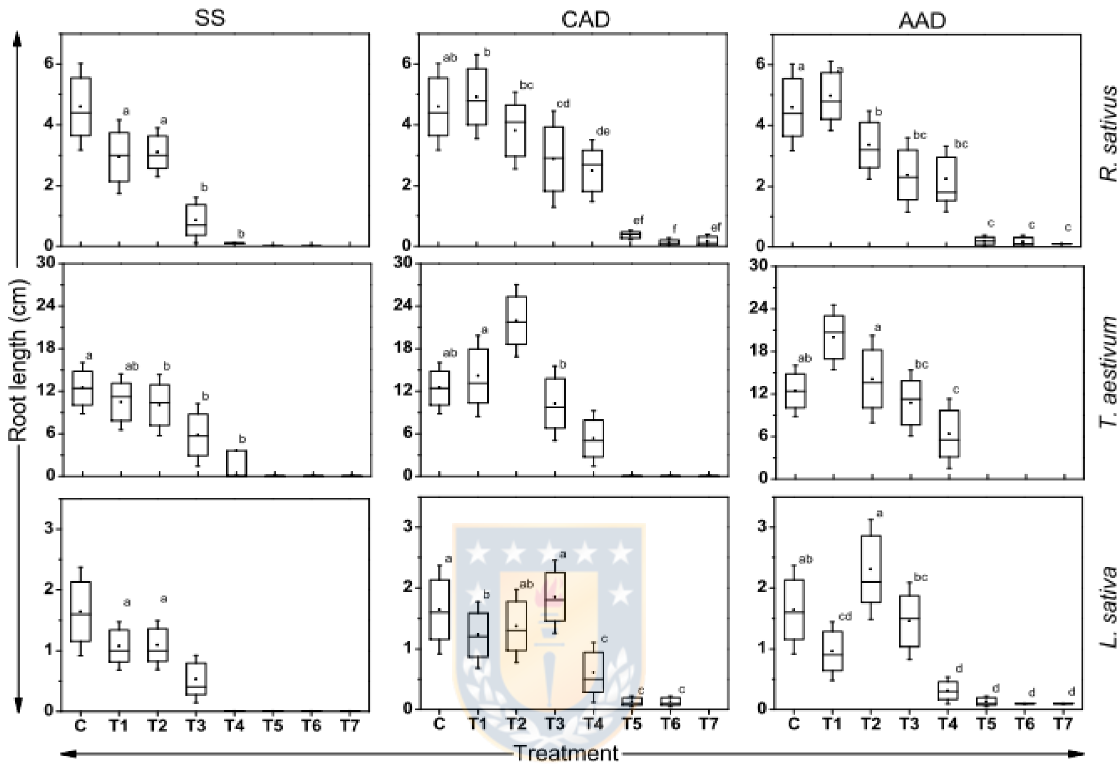

Figure 2. Relative root length (cm) of plant tested (*R. sativus*, *T. aestivum*, and *L. sativa*) with different treatments (C: 0, T1: 5, T2: 50, T3: 100, T4: 250, T5: 500, T6: 750, and T7: 1000 g/kg) of sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) is presented with a matrix form, where column corresponds to the stabilization stage and row to the plant used. Treatment marked with the same letter, are not significantly different (Kruskal-Wallis *p>0.05*).

Moreover, Figure 3 shows the relative sprout length of *R. sativus*, *T. aestivum* and *L. sativa* for different application doses of SS, CAD, and AAD. The results showed positive effects of the different samples on this endpoint evaluated for doses below 100 g/kg. In the case of *R. sativus*, the sprout growth was stimulated for application doses of 5-50 g/kg corresponding to SS, CAD, and AAD with values of 1.2-3.0 cm, 1.5-4.4 cm, and 1.0- 2.9 cm, respectively. The positive effects were also observed for SS, CAD, and AAD in *L. sativa*. For application doses of 5-50 g/kg, 100 g/kg, and 5-

100 g/kg of the samples before named, the sprout growth was between 0.8-1.7 cm, 0.4-1.7 cm, and 0.5-1.6 cm, respectively. However, for *T. aestivum*, significant differences with respect to the control species were detected for the application doses of 50 g/kg corresponding to CAD and for the application doses of 5 and 50 g/kg corresponding to AAD ($p>0.05$). In these cases, the sprout growth varied between 1.5-7.6 cm and 1.8-7.0 cm, respectively.

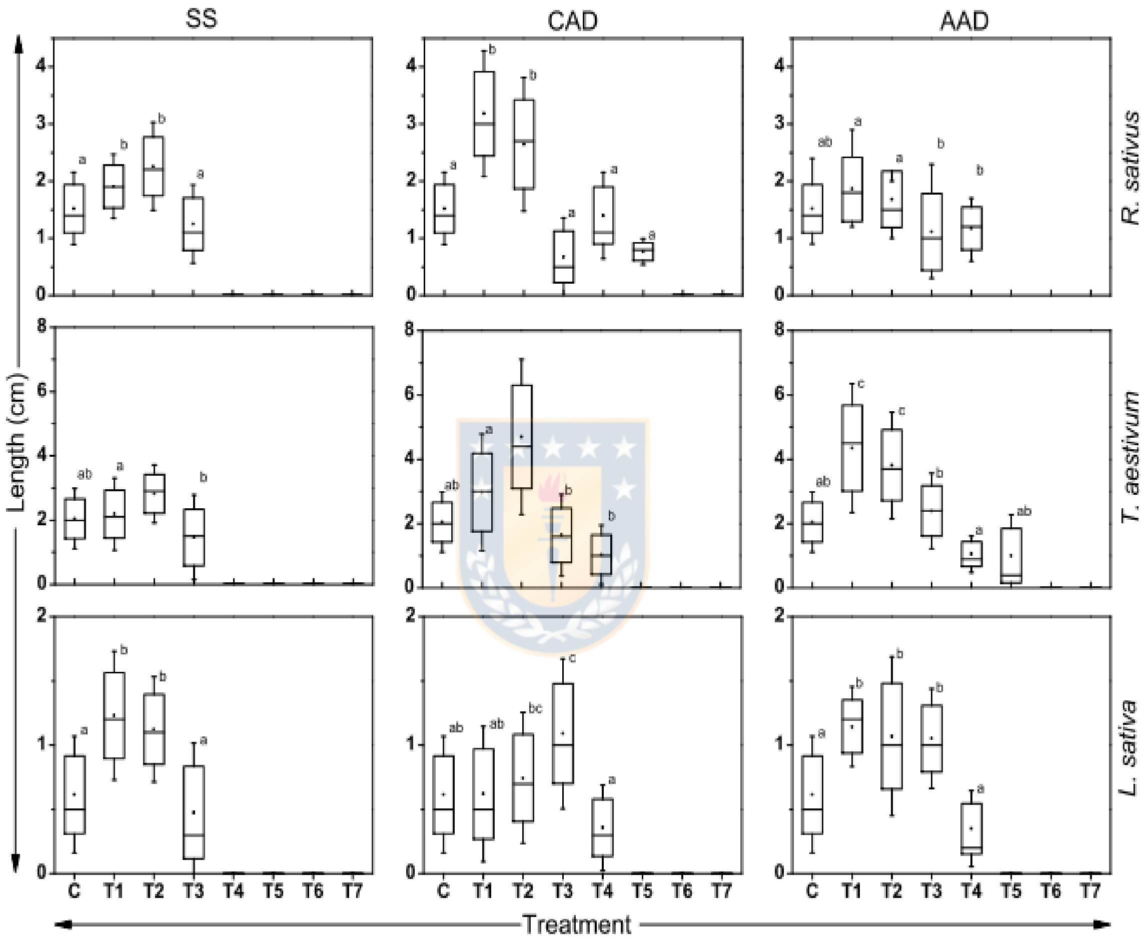

Figure 3. Relative sprout length (cm) of plant tested (*R. sativus*, *T. aestivum*, and *L. sativa*) with different treatments (C: 0, T1: 5, T2: 50, T3: 100, T4: 250, T5: 500, T6: 750, and T7: 1000 g/kg) of sewage sludge (SS), conventional anaerobic digestion (CAD) and advanced anaerobic digestion (AAD) is presented with a matrix form, where column corresponds to the stabilization stage and row to the plant used. Treatment, marked with the same letter, are not significantly different (Kruskal-Wallis $p>0.05$).

3.3. Effects of SS, CAD, and AAD on phytotoxicity index

Figure 4 shows the relative seed and root inhibition percentages of *R. sativus*, *T. aestivum* and *L. sativa* exposed to SS, CAD, and AAD. Even with the application dose of 0.5%, the use of different doses of SS caused the greatest inhibitions in both germination and root growth. Except for *T. aestivum* seed germination where doses of SS between 5-100 g/kg caused positive effects with percentages between 14-24%. In the cases of CAD and AAD, doses above 250 g/kg inhibited the germination of the three plants evaluated with values higher than 50%. The same positive effect was observed for *T. aestivum*, percentages between 33-38% and 28-33% were obtained for doses between 5-100 g/kg of CAD and AAD, respectively.

On the other hand, the results of this study can be compared with those presented in Table 2. For *L. sativa*, Kinney et al. (2012) reported germination inhibition that varied between 13-71%. In this study, similar ranges were obtained for CAD and AAD achieving values that fluctuated between 0-60% and 10-55%, respectively. Likewise, Ramírez et al. (2008) determined percentages of germination inhibition of 98, 23 and 74% for *B. rapa*, *L. perenne* and *T. pretense*, respectively, when the application dose was 151.4 g/kg. Moreover, for higher application doses (>151.4 g/kg), the inhibition percentages were 100%. Adamcová et al. (2016) showed that application doses of BS above 100 g/kg inhibited the germination of S. alba with percentages that varied between 94-100%. In this study, similar tendencies were observed for *R. sativus*, *T. aestivum* and *L. sativa* where application doses of BS (CAD and AAD) above 100 g/kg produced germination inhibitions between 53-87%, 47-100%, and 55-100%, respectively.

Other studies have been reported lower inhibition percentages or benefits effects. In the cases of *S. lycopersicum*, *L. perenne*, *P. ahip* and *B. rapa*, these values were close to 11, 3, 23 and 15%, respectively (Domene et al., 2011; Rossini-Oliva et al., 2017). For *R. sativus*, *T. aestivum* and *L. sativa*, the germination inhibition percentages fluctuated from 3 to 47%, from -38 to -28% and from 0 to 40%, respectively for application doses of CAD and AAD below 100 g/kg.

Table 2. Summary of the main parameters considered during phytotoxic assays for evaluating ecotoxicity of biosolids from anaerobic digestion stabilization process.

Soil	Stabilization process	Application doses	Endpoint	Plant	Inhibition in germination (%)	References
Natural	CAD	0, 1, 2, 3, 4%	G	*Lactuca sativa*	21.5, 56.99, 35.48, 70.97 13, 33, 47 ,67	(Kinney et al., 2012)
	CAD	0, 30, 60, 120 t/ha	G/B/GW	*Triticum aestivum* *Vicia sativa* *Brassica rapa*	3.67, 7.44, 3.77 0, 0, 0 25.93, 25.93, -11.11	(Carbonell et al., 2009)
	CAD	0, 3.65, 7.30, 14.6 t/ha	B	*Dactylis glomerata L* *Lolium perenne L.* *Trifolium repens L.*	-	(Ferreiro-Domínguez et al., 2011)
	CAD	0, 5.1, 10.2, 100%	B/BA	*Lolium multiflorum Lam.*	-	(Alvarenga et al., 2008)
	CAD/C	0, 2, 10%	G/B/P	*Solanum lycopersicum* *Lolium perenne L* *Pachyrhizus ahip*	11.96, 11.96 -3.30, 3.30 28.73, 22.99	(Rossini-Oliva et al., 2017)
	C/CAD	0, 1.2, 4, 20, 50 g/kg	G	*Brassica rapa L* *Lolium perenne L*	11.1, 11.1, 16.7, 11.1 10, 10, 15, 15 5.6, 16.7, 22.2, 22.2 5, 15, 20, 15	(Domene et al., 2011)

Table 2. Continued

Soil	Stabilization process	Application doses	Endpoint	Plant	Inhibition in germination (%)	References
Artificial	CAD	10, 25, 50, 100%	G/RL	*Sinapis alba L*	79.30, 96.69, 98.63, 100 70.45, 94.18, 99.64, 100 81.60, 97.63, 98.99, 100	(Adamcová et al., 2016)
	CAD	0, 25, 50, 75, 100%	GW	*Lepidium sativum L* *Hordeum vulgare L*	-	(Alvarenga et al., 2007)
	CAD/NS	0, 4, 8 g/kg	G/B	*Brassica rapa* *Avena sativa*	No interference in seed germination	(Moreira et al., 2008)
	AeD/CAD/C	0, 53.1, 75.2, 106.7, 151.4, 214.7, 304.6 g/kg	G/GW	*Brassica rapa* *Lolium perenne* *Trifolium pretense*	15, 44, 20, 98, 100, 100 3, 9, 11, 23, 100, 100 21, 38, 53, 74, 100, 100	(Ramírez et al., 2008)
		0, 3.0, 4.9, 8.1, 13.3, 21.8, 35.7 g/kg		*Brassica rapa* *Lolium perenne* *Trifolium pretense*	56, 83, 87, 91, 100, 100 44, 49, 53, 100, 100, 100 17, 25, 56, 82, 100, 100	
	AAD	0, 0.5, 5, 10, 25, 50, 75, 100%	G/RL/GW	*Raphanus sativus*	3, 27, 47, 53, 53, 67, 87 23, 20, 43, 60, 67, 63, 87	This study
				Triticum aestivum	-38, -33, -38, 47, 100, 100, 100 -28, -33, -33, 71, 90, 100, 100	
				Lactuca sativa	0, 10, 40, 60, 85, 85, 100 30, 10, 40, 55, 85, 85, 90	

Stabilization process: AeD: aerobic digestion, NS: non-stabilized, CAD: conventional anaerobic digestion, AAD: advanced anaerobic digestion, AD: anaerobic digestion, Dg: digestion not specify, C: compost.
Endpoint: G: germination, B: biomass, RL: root length, BA: bioaccumulation, GW: growth, P: pigment or chlorophyll related.

Regarding the inhibition of the root growth, the SS application caused inhibitory effects with percentages that varied between 32-97%, 15-71% and 34-68% for *R. sativus*, *T. aestivum*, and *L. sativa*, respectively (Figure 4). The same results were observed for application doses above 250 g/kg of CAD and AAD for *R. sativus* and *L. sativa* which presented inhibition percentages of the root growth higher than 45%. It is reported that BS from CAD had a high degree of toxicity with inhibition on the root growth of *Sinapis alba* with percentages that fluctuated between 70-100% for application doses between 100-1000 g/kg (Adamcová et al., 2016). However, stimulant effects on the root growth were determined for BS application rates of 5 g/kg for *R. sativus*, 5-50 g/kg for *T. aestivum*, 50 and 100 g/kg for *L. sativa* with percentages of -8%, -13 to -77% and -12 to -40%, respectively.

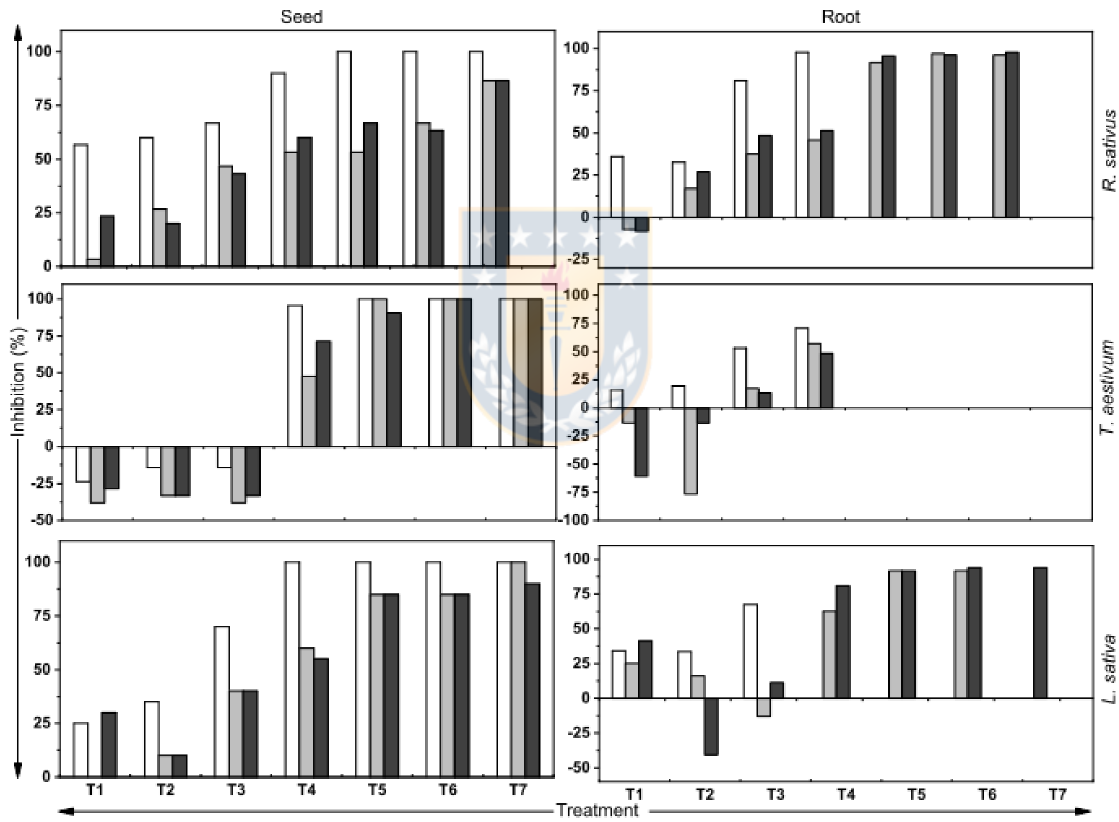

Figure 4. The relative seed and root inhibition (%) of plant tested (*R. sativus*, *T. aestivum* and *L. sativa*) with different treatments (C: 0, T1: 5, T2: 50, T3: 100, T4: 250, T5: 500, T6: 750, and T7: 1000 g/kg) of sewage sludge (SS, white square), conventional anaerobic digestion (CAD, gray square) and advanced anaerobic digestion (AAD, black square).

Figure 5 shows the germination index (GI) of *R. sativus*, *T. aestivum* and *L. sativa* for different application doses of SS, CAD, and AAD. In the case of *R. sativus*, high toxic effects with GI ≤ 50% were observed for application doses below 100 g/kg of SS, CAD, and AAD. Regarding the effects of CAD on this plant tested, application doses of 5 g/kg achieved GI ≥ 100% which indicated a positive influence on seeds germination. However, for application dose of 50 g/kg, moderated toxicity with GI between 50 and 80% was reported. The same tendency was observed for the same application doses of AAD. For *T. aestivum*, GI ≥ 100% were achieved with application doses between 5-100 g/kg of CAD and AAD which indicated benefit effects. For *L. sativa*, a positive effect on seeds germination was determined for application doses of 50 g/kg of AAD with GI ≥ 100%. Ours previous studies of the phytotoxicity of elutriated from BS (CAD and AAD) showed that AAD elutriated had a beneficial effects or no presence of phytotoxic substances for the same tested plants and conclude that the application of a pretreatment (sequential ultrasound and low-thermal hydrolysis) reduce the phytotoxicity of the sewage biosolids-elutriated with GI up to 90% (Venegas et al., 2018).

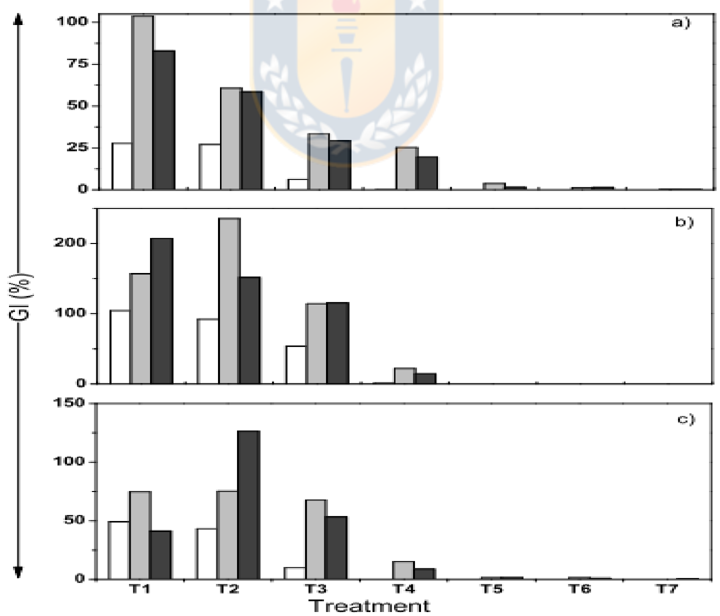

Figure 5. Germination Index (%) relative to plant tested (a) *R. sativus*, b) *T. aestivum* and c) *L. sativa*) with different treatments (C: 0, T1: 5, T2: 50, T3: 100, T4: 250, T5: 500, T6: 750, and T7: 1000 g/kg) of sewage sludge (SS, white square), conventional anaerobic digestion (CAD, gray square) and advanced anaerobic digestion (AAD, black square).

Table 3 shows the EC_{50} toxicity values for germination, root length and sprout length for different plants and samples evaluated. In agreement with the results in table 3, in this study, the bioassays with SS have the highest toxic effects for all the endpoints in the three plants tested with the lowest EC_{50} value, 19 g /kg for the sprout length of *R. sativus*. The phytotoxicity assay with CAD and AAD biosolids had similar EC_{50} values. The most toxic effect of the BS was related with CAD samples and was on the sprout length of *R. sativus* and *T. aestivum* with values of 97 and 91 g/kg, respectively. The highest EC_{50} values 146, 222 and 230 g/kg for the root growth of *R. sativus*, for the sprout length of *L. sativa* and for *T. aestivum* germination, respectively. Ramírez et al. (2008) reported EC_{50} values for the germination (table 3) between 21-82 g/kg for BS from anaerobic digestion with and without post-treatment with thermally dried. These values were more toxic than those reported in this study with values for SS and BS (CAD and AAD) samples between 89-163 g/kg and 102-230 g/kg, respectively. Similar results were observed for the sprout length. In this case, Ramírez et al. (2008) showed values between 23-80 g/kg whereas, in this study, these values varied between 19-58 g/kg and between 91-222 g/kg for plants exposed to SS and BS (CAD and AAD), respectively.

Table 3. EC_{50} toxicity values for germination, root length and sprout length as g/kg, with their standard error in parentheses.

Endpoint		**Sample from**		
		SS	**CAD**	**AAD**
Germination EC_{50} (g/kg)	***R. sativus***	163 (7)	201 (49)	211 (50)
	T. aestivum	158 (16)	230 (10)	199 (17)
	L. sativa	89 (2)	164 (18)	173 (40)
Root length EC_{50} (g/kg)	***R. sativus***	88 (10)	146 (27)	102 (23)
	T. aestivum	86 (1)	115 (51)	116 (6)
	L. sativa	71 (11)	207 (61)	118 (42)
Sprout length EC_{50} (g/kg)	***R. sativus***	19 (1)	97 (39)	186 (48)
	T. aestivum	37 (175)	91 (20)	129 (16)
	L. sativa	58(3)	222 (130)	196 (9)

SS: sewage sludge, CAD: conventional anaerobic digestion, AAD: advanced anaerobic digestion.

3.4. Relation of operational conditions, physicochemical characteristic and the phytotoxicity assay

The use of AAD allows reaching a better anaerobic digestion performance reaching an increasing in biogas production and a reduction in solids content (Neumann et al., 2016). The sequential pre-treatment used in this study allows an increase of 19% in methane yield and a 33% reduction in total solids. Also, a decrease between 19-73% in the concentration of tonalide, galaxolide, triclosan, butylated hydroxytoluene, phenanthrene, pyrene, 4-nonylphenol, and 17β-ethynylestradiol in the BS (AAD) was achieved, this compared to the concentration in BS from CAD (Reyes-Contreras et al., 2018). However, the average concentration of this micropollutant rise in the BS compared with the parental SS, between 31-360% for CAD, and 8-274% for AAD, with concentration in the range of 0.29-59.41 µg/g and 0.12-43.99 µg/g for CAD and AAD, respectively (Reyes-Contreras et al., 2018).

Ecotoxicity data, phytotoxicity among them, associated to BS micropollutants are very scarce (Richter et al., 2016). From the few studies carry on, Richter et al. (2016) found that for BS (CAD) at application rate of 5 ton/ha evaluated with *Brassica napus*, EC_{50} values for ketoconazole and BDDA (benzododecinium chloride) tent to be higher in the assay with BS compared to the assay without them. Peña et al. (2014), reported that BS application significantly reduce pesticides phytotoxicity uptake into rygrass. Prosser et al. (2014), found that BS from CAD at 12-29 ton/ha significantly reduced triclosan uptake into plants roots. This behaviors in the reduction in the toxicity, may be related with the sequestration processes that could reduce the bioavailability of the micropollutants (Richter et al., 2016; Wu et al., 2009).

Comparing the concentration of micropollutants evaluated by Richter et al. (2016) and Peña et al. (2014), 62.5-1000 µg/g of ketoconazole and 7.6-46.6 µg/g triclosan, respectively; with the range in the BS (CAD and AAD) used in this study 0.12-59.41 µg/g of all micropollutants evaluated and 2.47-9.28 µg/g of triclosan. The concentration of micropollutants in this study are lower than the other studies before mentioned and considering the possible effect of sequestration of the micropollutants in the BS, the effect of the micropollutants in the phytotoxicity assay could be considered insignificant.

The metal content of the BS (CAD and AAD) used in these study has been determinate in previous research by Venegas et al. (2018), were an increase Cu and Zn concentration was reported for both BS. These behaviors in metal concentration have been related to the degradation of organic and inorganic matter during the digestion (Carballa et al., 2009; Chipasa, 2003). Zinc and copper are essentials elements, are metalloenzimes for enzyme such as anhydrases, dehydrogenases, oxidases and peroxidases (Kopittke and Menzies, 2006; Rout and Das, 2009). Zinc plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis (Rout and Das, 2009); copper plays an important role in CO_2 assimilation, ATP synthesis, also is component of various proteins of the photosynthetic system and of the respiratory electron transport chain (Yadav, 2010).

Metal content of BS, particularly Zn and Cu concentration, has not been related to phytotoxicity of BS from different stabilization processes (CAD, aerobic digestion, stabilization pond) (Fuentes et al., 2004; Wollan et al., 1978). For *Lolium perenne* at concentration between 990-1050 mg/kg and 2213-5650 mg/kg of Zn and Cu, respectively, (Wollan et al., 1978) and for *Hordeum vulgare* and *Lepidium sativum* at concentrations between 458-871 mg/kg and 146-337 mg/kg of Zn and Cu, respectively, (Fuentes et al., 2004) the phytotoxicity were associated to the influence of nitrogenous compounds as ammonia and ammonium and not to the metal content. Metal phytotoxicity can result only if metal can move from the soil to the roots systems (Rout and Das, 2009), so considering the low extractability of metal from BS (Fuentes et al., 2006), and that Zn and Cu concentration in this study were lower than the reported in other research, it is likely that the concentration of metals is not the main cause of the negative effects on tested plants growth.

Due the metabolic route of anaerobic digestion process, organic nitrogen and NH_4^+-N are the main forms of nitrogen present in the BS (Yang et al., 2018). The NH_4^+-N concentration was 29% greater in AAD than in CAD. That agree with the increase of the solubilization of organic matter, including nitrogen compounds, due the application of a pre-treatment step (Carvajal et al., 2013; Dhar et al., 2012; Neumann et al., 2018). In phytotoxicity assay of BS from anaerobic digestion is common attributed a negative effect on seed germination to the release of NH_4^+-N (Fuentes et al., 2006).

For *Lepidium sativum* concentration under 2000 mg/kg of NH_4^+-N shows GI >80 (Tiquia, 2010). Similar results were found in this study, where the GI of the BS (CAD and AAD) indicated benefit effects (GI ≥ 100) or no presence of phytotoxicity (100 > GI ≥ 80) for application doses between 5-100 g/kg. At these application rate of BS the content of NH_4^+-N vary between 87-1750 mg/kg for CAD, and 113-2260 mg/kg for AAD.

4 Conclusions

The application of SS samples on soil had greater toxic effects in the different endpoints with germination inhibition percentages above 50% for application doses of 250 g/kg. Regarding GI values, benefit effects (GI ≥100) or no presence of phytotoxicity (100 > GI ≥ 80) were observed for application doses of BS (CAD and AAD) between 5-100 g/kg. Between BS evaluated, CAD samples were slightly more beneficial than those of AAD with an increased-on GI value between 4-20% for *R. sativus* with application doses between 5-50 g/kg. In the case of *T. aestivum* and *L. sativa*, these differences were 35% and 45% for application doses of 50 g/kg and 5 g/kg, respectively. These results were corroborated with EC_{50} values. For all plants tested with CAD, the most toxic value was 91 g/kg with an average of 164 g/kg for the endpoints evaluated. Likewise, for the endpoints and plants evaluated with AAD, these values were 102 g/kg with an average of 159 g/kg. Regarding the plant development, *L. sativa* was the most sensible plant with germination and root growth inhibitions between 0-100% and -40 and 94%, respectively.

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References

Adamcová, D., Vaverková, M., Břoušková, E., 2016. The toxicity of two types of sewage sludge from wastewater treatment plant for plants in Czech Republic. J. Ecol. Eng. 17, 33–37. https://doi.org/10.12911/22998993/62283

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774–1781. https://doi.org/10.1016/j.chemosphere.2008.04.042

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., Cunha-Queda, A.C., Duarte, E., Vallini, G., 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33, 505–513. https://doi.org/10.1016/j.envint.2006.11.006

Alvarenga, P., Palma, P., Mourinha, C., Farto, M., Dôres, J., Patanita, M., Cunha-Queda, C., Natal-da-Luz, T., Renaud, M., Sousa, J.P., 2017. Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. Waste Manag. 61, 582–592. https://doi.org/10.1016/j.wasman.2017.01.004

Antilén, M., Silva, K., Acevedo, S., Amiama, F., Faúndez, M., Knicker, H., Pizarro, C., 2014. Characterization of humic acids extracted from biosolid amended soils. J. Soil Sci. Plant Nutr. 14, 1005–1020.

APHA, AWWA, WEF, 2012. Standard Methods for examination of water and wastewater, 22nd ed. American Public Health Association, Washington.

Appels, L., Degrève, J., Van der Bruggen, B., Van Impe, J., Dewil, R., 2010. Influence of low temperature thermal pre-treatment on sludge solubilisation, heavy metal release and anaerobic digestion. Bioresour. Technol. 101, 5743–8. https://doi.org/10.1016/j.biortech.2010.02.068

Arias, F., Sen, T.K., 2009. Removal of zinc metal ion (Zn2+) from its aqueous solution by kaolin clay mineral: A kinetic and equilibrium study. Colloids Surfaces A Physicochem. Eng. Asp. 348, 100–108. https://doi.org/10.1016/j.colsurfa.2009.06.036

Braguglia, C.M., Coors, A., Gallipoli, A., Gianico, A., Guillon, E., Kunkel, U., Mascolo, G., Richter, E., Ternes, T. a., Tomei, M.C., Mininni, G., 2015. Quality assessment of digested sludges produced by advanced stabilization processes. Environ. Sci. Pollut. Res. 22, 7216–7235. https://doi.org/10.1007/s11356-014-3090-6

Carballa, M., Omil, F., Lema, J.M., 2009. Influence of different pretreatments on anaerobically digested sludge characteristics: Suitability for final disposal. Water. Air. Soil Pollut. 199, 311–321. https://doi.org/10.1007/s11270-008-9880-z

Carbonell, G., Pro, J., Gómez, N., Babín, M.M.M., Fernández, C., Alonso, E., Tarazona, J.V. V., 2009. Sewage sludge applied to agricultural soil: Ecotoxicological effects on representative soil organisms. Ecotoxicol. Environ. Saf. 72, 1309–1319. https://doi.org/10.1016/j.ecoenv.2009.01.007

Carvajal, A., Peña, M., Pérez-Elvira, S., 2013. Autohydrolysis pretreatment of secondary sludge for anaerobic digestion. Biochem. Eng. J. 75, 21–31. https://doi.org/10.1016/j.bej.2013.03.002

Chipasa, K.B., 2003. Accumulation and fate of selected heavy metals in a biological wastewater treatment system. Waste Manag. 23, 135–143. https://doi.org/10.1016/S0956-053X(02)00065-X

Dhar, B.R., Nakhla, G., Ray, M.B., 2012. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. Waste Manag. 32, 542–549. https://doi.org/10.1016/j.wasman.2011.10.007

Domene, X., Solà, L., Ramírez, W., Alcañiz, J.M., Andrés, P., 2011. Soil bioassays as tools for sludge compost quality assessment. Waste Manag. 31, 512–522. https://doi.org/10.1016/j.wasman.2010.10.013

Ferreiro-Domínguez, N., Rigueiro-Rodríguez, A., Mosquera-Losada, M.R.R., 2011. Response to sewage sludge fertilisation in a Quercus rubra L. silvopastoral system: Soil, plant biodiversity and tree and pasture production. Agric. Ecosyst. Environ. 141, 49–57. https://doi.org/10.1016/j.agee.2011.02.009

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M.I., Ortuño, J.F., Meseguer, V.F., 2004. Phytotoxicity and heavy metals speciation of stabilised sewage sludges. J. Hazard. Mater. 108, 161–169. https://doi.org/10.1016/j.jhazmat.2004.02.014

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M.I., Pérez-Marín, A.B., Ortuño, J.F., Meseguer, V.F., 2006. Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. Environ. Pollut. 143, 355–360. https://doi.org/10.1016/j.envpol.2005.11.035

Gerber, M.D., Lucia, T., Correa, L., Neto, J.E.P., Correa, É.K., 2017. Phytotoxicity of effluents from swine slaughterhouses using lettuce and cucumber seeds as bioindicators. Sci. Total Environ. 592, 86–90. https://doi.org/10.1016/j.scitotenv.2017.03.075

Kinney, C.A., Campbell, B.R., Thompson, R., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Hay, A.G., 2012. Earthworm bioassays and seedling emergence for monitoring toxicity, aging and bioaccumulation of anthropogenic waste indicator compounds in biosolids–amended soil. Sci. Total Environ. 433, 507–515. https://doi.org/10.1016/j.scitotenv.2012.06.097

Kopittke, P.M., Menzies, N.W., 2006. Effect of Cu Toxicity on Growth of Cowpea (Vigna unguiculata). Plant Soil 279, 287–296. https://doi.org/10.1007/s11104-005-1578-z

Moreira, R., Sousa, J.P.P., Canhoto, C., 2008. Biological testing of a digested sewage sludge and derived composts. Bioresour. Technol. 99, 8382–8389. https://doi.org/10.1016/j.biortech.2008.02.046

Neumann, P., Barriga, F., Álvarez, C., González, Z., Vidal, G., 2018. Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound–thermal (55 °C) pre-treatment. Bioresour. Technol. 262, 42–51. https://doi.org/10.1016/j.biortech.2018.03.057

Neumann, P., González, Z., Vidal, G., 2017. Sequential ultrasound and low-temperature thermal pretreatment: Process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. Bioresour. Technol. 234, 178–187. https://doi.org/10.1016/j.biortech.2017.03.029

Neumann, P., Pesante, S., Venegas, M., Vidal, G., 2016. Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Rev. Environ. Sci. Bio/Technology 15, 173–211. https://doi.org/10.1007/s11157-016-9396-8

OECD/OCDE, 2006. Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD. https://doi.org/10.1787/9789264070066-en

OECD/OCDE, 1984. Test No. 207: Earthworm, Acute Toxicity Tests, OECD Guidelines for the Testing of Chemicals, Section 2. OECD. https://doi.org/10.1787/9789264070042-en

Oleszczuk, P., 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69, 496–505. https://doi.org/10.1016/j.ecoenv.2007.04.006

Papa, M., Foladori, P., Guglielmi, L., Bertanza, G., 2017. How far are we from closing the loop of sewage resource recovery? A real picture of municipal wastewater treatment plants in Italy. J. Environ. Manage. 198, 9–15. https://doi.org/10.1016/j.jenvman.2017.04.061

Peña, A., Mingorance, M.D., Guzmán, I., Sánchez, L., Fernández-Espinosa, A.J., Valdés, B., Rossini-Oliva, S., 2014. Protecting effect of recycled urban wastes (sewage sludge and wastewater) on ryegrass against the toxicity of pesticides at high concentrations. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2014.04.002

Prosser, R.S., Lissemore, L., Topp, E., Sibley, P.K., 2014. Bioaccumulation of triclosan and triclocarban in plants grown in soils amended with municipal dewatered biosolids. Environ. Toxicol. Chem. 33, 975–984. https://doi.org/10.1002/etc.2505

Ramírez, W.A.A., Domene, X., Ortiz, O., Alcañiz, J.M.M., 2008. Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresour. Technol. 99, 7168–7175. https://doi.org/10.1016/j.biortech.2007.12.072

Reyes-Contreras, C., Neumann, P., Barriga, F., Venegas, M., Domínguez, C., Bayona, J.M.J.M., Vidal, G., 2018. Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization. Environ. Technol. 1–8. https://doi.org/10.1080/09593330.2018.1534892

Richter, E., Roller, E., Kunkel, U., Ternes, T.A., Coors, A., 2016. Phytotoxicity of wastewater-born micropollutants – Characterisation of three antimycotics and a cationic surfactant. Environ. Pollut. 208, 512–522. https://doi.org/10.1016/J.ENVPOL.2015.10.024

Roig, N., Sierra, J., Nadal, M., Martí, E., Navalón-Madrigal, P., Schuhmacher, M., Domingo, J.L., 2012. Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants. Sci. Total Environ. 425, 99–109. https://doi.org/10.1016/j.scitotenv.2012.03.018

Rossini-Oliva, S., Mingorance, M.D., Peña, A., 2017. Effect of two different composts on soil quality and on the growth of various plant species in a polymetallic acidic mine soil. Chemosphere 168, 183–190. https://doi.org/10.1016/j.chemosphere.2016.10.040

Rout, G.R., Das, P., 2009. Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc, in: Sustainable Agriculture. Springer Netherlands, Dordrecht, pp. 873–884. https://doi.org/10.1007/978-90-481-2666-8_53

Tiquia, S.M., 2010. Reduction of compost phytotoxicity during the process of decomposition. Chemosphere. https://doi.org/10.1016/j.chemosphere.2010.02.040

Tiwary, A., Williams, I.D., Pant, D.C., Kishore, V.V.N., 2015. Emerging perspectives on environmental burden minimisation initiatives from anaerobic digestion technologies for community scale biomass valorisation. Renew. Sustain. Energy Rev. 42, 883–901. https://doi.org/10.1016/j.rser.2014.10.052

Venegas, M., Leiva, A.M., Vidal, G., 2018. Influence of Anaerobic Digestion with Pretreatment on the Phytotoxicity of Sewage Sludge. Water, Air, Soil Pollut. 229, 381. https://doi.org/10.1007/s11270-018-4025-5

Wollan, E., Davis, R.D., Jenner, S., 1978. Effects of sewage sludge on seed germination. Environ. Pollut. https://doi.org/10.1016/0013-9327(78)90037-X

Wu, C., Spongberg, A.L., Witter, J.D., 2009. Sorption and biodegradation of selected antibiotics in biosolids. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 44, 454–461. https://doi.org/10.1080/10934520902719779

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African J. Bot. 76, 167–179. https://doi.org/10.1016/j.sajb.2009.10.007

Yang, Y., Zhang, Y., Li, Y., Zhao, H., Peng, H., 2018. Nitrogen removal during anaerobic digestion of wasted activated sludge under supplementing Fe(III) compounds. Chem. Eng. J. 332, 711–716. https://doi.org/10.1016/J.CEJ.2017.09.133

CAPÍTULO VIII

DISCUSIÓN

Toda actividad humana requiere del uso y consumo de agua potable, una vez utilizada se generan aguas servidas y durante el tratamiento de estas la generación de lodos sanitarios (LS), tal como se mostró en la Figura 1 del Capítulo II. Los LS pueden ser tratados por medio de digestión anaerobia convencional (DAC) o avanzada (DAA), tal cómo se presentó en la sección 2 del Capítulo II. Una vez estabilizados los LS, denominados biosólidos (BS), tienen el potencial de ser aplicado como mejorador de suelos. Pero debido a sus mismas características, la aplicación de BS tiene una serie de implicaciones, las cuales se discutirán en las secciones siguientes.

La Figura 1 presenta la propuesta de un sistema de economía circular en el sector sanitario. Donde la estabilización de los LS por medio de digestión anaerobia presenta la oportunidad de utilizar un subproducto del tratamiento de las aguas servidas para la obtención de energía, así como el uso de los BS como mejoradores de suelo, es decir, se puede dar un cambio de paradigma de “tratamiento para disponer” por “tratamiento para reusar” incorporando al sector sanitario cada vez más a la ideología de la economía circular.

Figura 1. Propuesta de un sistema de economía circular en el sector sanitario.

Pero para poder cerrar el ciclo del sector sanitario de forma segura se debe evaluar la calidad del BS considerando parámetros fisicoquímicos, microbiológicos y fitotoxicológicos. Por ello el objetivo de esta tesis fue determinar la influencia de la digestión anaerobia convencional y avanzada en la composición y fitotoxicidad de los BS obtenidos. La aplicación de un pre-tratamiento secuencial de ultrasonido seguido de un tratamiento térmico a baja temperatura, produce la disrupción física de las células liberando las enzimas endógenas durante el ultrasonido y posteriormente en la etapa térmica del pre-tratamiento estimular una reactivación de la actividad enzimática endógena, así como una hidrólisis térmica. Lo que resulta en incrementos de 458 – 1030% y 252 – 674% en la concentración soluble de carbohidratos y proteínas, respectivamente (Neumann et al., 2017) y aumentos en el rendimiento de metano de 30%. Una mayor discusión del efecto del pre-tratamiento en la digestión anaerobia se presenta en la sección 1 de este Capítulo.

Considerando que los BS generados por la DAC y DAA van a ser aplicados como remediadores, se evaluó la fitotoxicidad directa e indirecta. Esto debido a que los ensayos de fitotoxicidad son herramientas útiles para evaluar residuos a ser aplicados en el suelo. Para este propósito se realizaron bioensayos con semillas de *Lactuca sativa*, *Raphanus sativus*, y *Triticum aestivum*. En la sección 3 y 4 se abordan a profundidad los efectos de los BS generados en la fitotoxicidad indirecta y directa respectivamente. En la sección 5 se profundiza en las implicaciones de la aplicación benéfica de BS.

1. Influencia del pre-tratamiento en la digestión anaerobia y en las características del biosólido

El primer objetivo específico del estudio fue determinar la influencia de la DAC y DAA en el desempeño de la digestión y en las características fisicoquímicas y microbiológicas de los BS generados. Para evaluar la influencia del pre-tratamiento secuencial en el desempeño de la digestión anaerobia se monitorearon parámetros operacionales como transformación de materia orgánica (demanda química de oxígeno, amonio, sólidos totales y volátiles), producción de biogás y rendimiento de generación de metano. También se monitoreó el LS utilizado, así como los BS generados por ambos reactores, DAC y DAA.

En la Figura 2 se presenta el porcentaje de eliminación de la demanda química de oxígeno (DQO) y el rendimiento en la generación de metano que presentaron los reactores durante el periodo de operación de aproximadamente 200 días. El reactor con DAA mostró un promedio de eliminación de DQO de 38.55% y rendimiento promedio de metano de 216.31 mL/g SV_0 (sólidos volátiles iniciales). Esto corresponde a aumentos en la eliminación de la DQO y en el rendimiento de metano de un 19.76% y 30.24%, respectivamente, en comparación con el reactor DAC. Investigaciones sobre este mismo pre-tratamiento secuencial reportan aumentos en el rendimientos de metano entre 14-50% y eliminaciones de DQO entre 17-28% mayores que la DAC (Dhar et al., 2012; Neumann et al., 2018; Şahinkaya and Sevimli, 2013). El aumento del rendimiento de metano y mayores eliminaciones de DQO se debe a que el pre-tratamiento secuencial incrementa la solubilización de la DQO en factores entre 3.5-17.1%, cumpliendo el propósito de facilitar la etapa hidrolítica de la digestión anaerobia y mejorar el desempeño de esta.

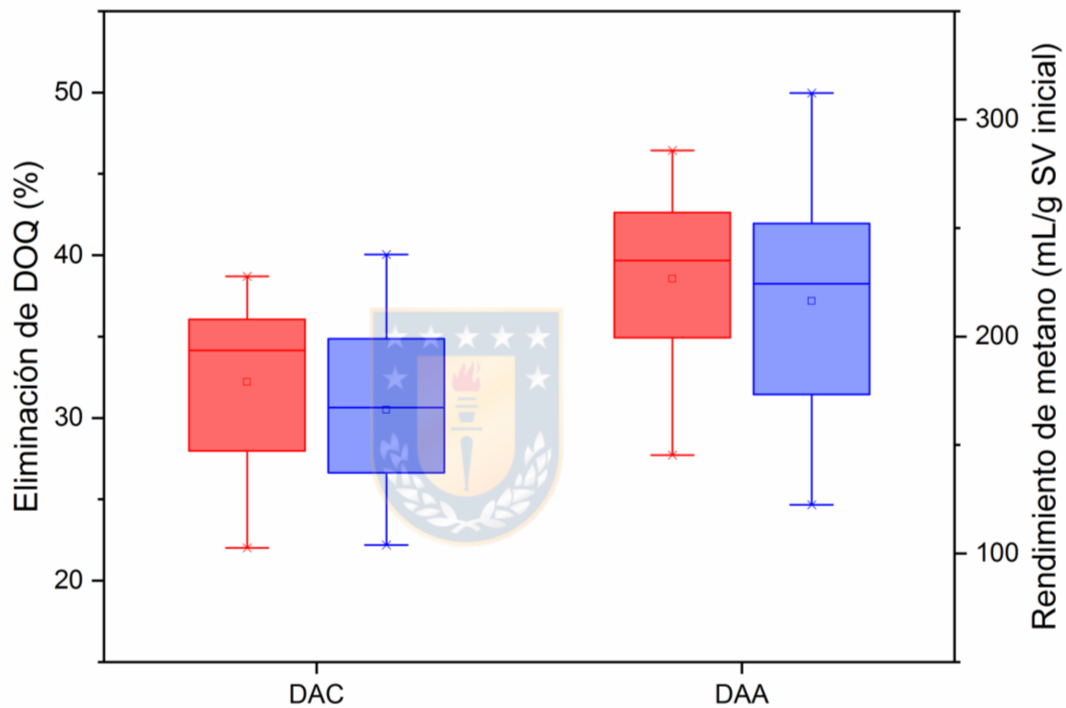

Figura 2. Eliminación de la demanda química de oxígeno (DQO, barras rojas) y el rendimiento de metano (barras azules) de los sistemas de digestión anaerobia convencional (DAC) y avanzada (DAA).

En los capítulos anteriores (IV, VI y VII), se ha discutido el efecto de la DAC y la DAA sobre las características fisicoquímicas de los BS generados, así como una descripción de las características de los LS parentales. En la Tabla 1 se muestra un resumen de las principales características fisicoquímicas de los BS de DAC y DAA. El pH de los BS mostró una tendencia hacia la neutralidad con promedios de 7.23 y 7.43 para los BS de DAC y DAA, respectivamente. Valores que se encuentran dentro de los reportados en la literatura para sistemas de digestión anaerobia, 6.50-7.54 (Alvarenga et al., 2008; Carbonell et al., 2009; Ferreiro-

Domínguez et al., 2011). En la sección 4 de la discusión se profundiza sobre la relación entre los valores de estos parámetros y la fitotoxicidad.

El contenido de potasio y fósforo en los BS también se encontraron dentro de los rangos reportados en la literatura 1.3-2.9 g/kg y 19.3-34.9 g/kg respectivamente (Alvarenga et al., 2008; Ferreiro-Domínguez et al., 2011; Moreira et al., 2008; Ramírez et al., 2008). Sin embargo, se reporta un contenido de fósforo en los BS de DAA con concentraciones aproximadamente 55% mayor (p > 0.05), lo cual se asocia al efecto de la lisis celular debido al pre-tratamiento, que produce incrementos de 458 – 1030% y 252 – 674% en la concentración soluble de carbohidratos y proteínas, respectivamente (Neumann et al., 2017). Aumentando la disponibilidad de algunos compuestos como el fósforo (Reyes-Contreras et al., 2018).

En el caso del contenido de nitrógeno total no se encontraron diferencias significativas (p > 0.05) entre los BS de DAC y DAA. El contenido de metales se encuentra dentro los límites permitidos por el Decreto Supremo 04, legislación chilena para aplicación de BS en suelo (MINSEGPRES, 2009). La estabilización de los BS se refleja en remociones de SV del 41% y 48%, así como en razones de SV/ST de 0.7 y 0.6 para CAD y AAD respectivamente.

Tabla 1. Caracterización fisicoquímica de los biosólidos de digestión anaerobia convencional y avanzada.

Parámetro	Unidades	CAD	AAD
SV/ST	-	0.6	0.5
DQO	g/L	174.95±55.17	150.25±64.28
pH	-	7.23±0.07	7.43±0.13
Humedad	%	88.85±0.42	87.19±0.95
MO	%	67.89±0.23	64.54±0.19
CE	mS/cm	2.83±0.18	2.45±0.65
COT	%	53.00±0.01	69.00±0.01
Pt	g/kg	18.9±0.7	34.9±1.6
K	g/kg	1.89±0.07	2.65±0.07
TN	g/kg	62.130±0.5	65.31±14.3
$N\text{-}NH_4^+$	g/kg	17.5±0.48	22.6±0.48
Cu	mg/kg	288	455
Ni	mg/kg	<1	<1
Zn	mg/kg	656	702
Pb	mg/kg	<3	<3
Hg	mg/kg	1.28	1.94

CE: conductividad eléctrica; Nt: nitrógeno total; NTK: Nitrógeno total Kjeldahl; Pt; fósforo total; SV: sólidos volátiles; ST: sólidos totales; DQO: demanda química de oxígeno; DAC: digestión anaerobia convencional; DAA: digestión anaerobia avanzada.

Respecto al efecto de la DAC y la DAA sobre la concentración de patógenos en la Tabla 2 se muestra una comparativa de la remoción de patógenos obtenida en esta investigación y la reportada en la literatura. El efecto del pre-tratamiento sobre la remoción de patógenos está asociada a que la implosión de las burbujas durante la cavitación debido a la aplicación del ultrasonido generan esfuerzos de corte sobre los microorganismos, mientras que la aplicación del pre-tratamiento térmico contribuye a la inactivación de patógenos debido al efecto de la temperatura en la desnaturalización de proteínas (Carballa et al., 2009; Harris and McCabe, 2015).

En este estudio la DAC logró reducciones logarítmicas para coliformes fecales, coliformes totales y colífagos somáticos de 2.03, 1.78 y 0.37; respectivamente. Mientras, que la DAA logró remociones de 2.15, 2.05 y 1.16 para los mismos parámetros. Para DAC se reportan remociones entre 2.1-2.8; 2.3-2.8 y 0.6-0.7 para coliformes totales, coliformes fecales y colífagos somáticos, respectivamente; mientras que para la DAA 2.4-3.1 y 1.5 para coliformes totales/fecales y colífagos somáticos, respectivamente (Levantesi et al., 2015). El hecho de que la DAA presente mayores remociones de agentes patógenos que la DAC, está ligado al efecto del pre-tratamiento. Los efectos hidrolíticos debidos a la fuerza de corte producido por la etapa de ultrasonido y a la desnaturalización de enzimas debido a la etapa térmica, también afectan a los agentes patógenos lo que genera una mayor remoción de estos en el proceso de DAA.

Tabla 2. Efecto de la digestión anaerobia convencional y avanzada en la concentración de patógenos.

Muestra		Coliformes fecales NMP/g ST	Coliformes totales NMP/g ST	Colífagos somáticos UFC/g ST
Literatura	LS	-	$1.2x10^{2}$-$2.4x10^{7}$	$8.9x10^{2}$-$8.1x10^{6}$
	DAC	$1.9x10^{4}$-$7.19x10^{7}$	$1.1x10^{2}$-$6.1x10^{9}$	$6.0x10^{1}$-$3.8x10^{6}$
	DAA	-	$9.0x10^{-2}$-$2.5x10^{5}$	$4.5x10^{2}$-$3.8x10^{5}$
Este estudio	LS	$4.78x10^{9}$	$4.78x10^{9}$	$5.81x10^{6}$
	DAC	$4.50x10^{5}$	$7.86x10^{5}$	$2.45x10^{6}$
	DAA	$3.40x10^{5}$	$4.21x10^{5}$	$4.02x10^{5}$

LS: lodo sanitario; DAC: digestión anaerobia convencional; DAA: digestión anaerobia avanzada; NMP: número más probable; ST: sólidos totales; UFC: unidades formadoras de colonia.
Referencias: (Bonomo et al., 2016; Carballa et al., 2009; Carmen Antolín et al., 2010; Corrêa Martins et al., 2016; Nafez et al., 2015; Renaud et al., 2017; Ros et al., 2015)

Si bien estos resultados de remoción son comparables con procesos de digestión reportados (Tabla 2), la densidad de coliformes fecales obtenidas es superior para que ambos BS sean clasificados como Clase A (10^3 NMP/g ST; MINSEGPRES 2009), pero si logran ser clasificados como Clase B ($2x10^6$ NMP/g ST;

MINSEGPRES 2009). Esto estaría relacionado con la elevada presencia de dichos microorganismos en las muestras de LS parental (4.8x10^9 NMP/g ST).

Respeto al efecto de los procesos de digestión anaerobia en el contenido de metales en los BS, los resultados reportados en la literatura, muestran que la concentración de metales en los BS generados ya sea por DAC o DAA y la concentración en los LS parentales tienden a permanecer en el mismo rango. Sin embargo, se pueden presentar aumentos en algunos casos, tal como en los BS estudiados, los que presentaron aumentos en la concentración de Cu y de Zn, con concentraciones entre 1.7-2.7 y 4.5-4.8 veces mayores que los LS, y el pre-tratamiento resultó además en incrementos de 7 – 58% en la concentración de As, Cu, Hg y Zn. Esto se asocia a la mayor eliminación de materia orgánica durante la DAC y DAA (Neumann et al., 2018). Dicho comportamiento también se podría asociar con fenómenos de precipitación y acumulación en los reactores (Carballa et al., 2009).

2. Efecto del pre-tratamiento en la digestión anaerobia sobre la concentración de microcontaminantes en el biosólido generado

El segundo objetivo específico fue evaluar la influencia de la DAC y DAA en la concentración de microcontaminantes en los BS generados. En el capítulo IV se discute el efecto de la DAC y la DAA sobre la concentración de microcontaminantes. Mientras que el capítulo V se examina la literatura existente relacionada con la presencia y destino de los microcontaminantes durante el tratamiento de las aguas servidas, particularmente la estabilización de los LS con tecnología de DAC y DAA.

Evaluar el efecto de las tecnologías de estabilización de LS en la toxicocinética de microcontaminantes enfrenta muchos retos. Los LS son una matriz compleja y la determinación de microcontaminantes muchas veces enfrenta problemas respecto a las técnicas analíticas existentes para su determinación en este tipo de matrices. Además, factores metodológicos como dopar los LS, pueden conllevar a una mala interpretación toxicocinética ya que muchas veces sobrepasan las concentraciones encontradas en las PTAS (Artola-Garicano et al., 2003). A continuación, se discuten los resultados del efecto del pre-tratamiento en la concentración de microcontaminantes y se compara con lo reportado por la literatura considerando los retos antes mencionados.

El destino de los microcontaminantes durante el tratamiento de las aguas servidas depende de las propiedades fisicoquímicas del compuesto (peso molecular, hidrofobicidad, solubilidad en agua, pKa, biodegradabilidad), de las características de las aguas servidas y de los LS generados (pH, materia orgánica, concentración de cationes); parámetros operacionales de la planta de tratamiento (presencia o ausencia de sedimentar primario, tiempo de residencia hidráulica, tiempo de residencia de lodos, tipo de tratamiento secundario, carga volumétrica) y de la frecuencia de uso de los microcontaminantes (Carballa et al., 2008; Holbrook et al., 2002; Radjenović et al., 2009; Stasinakis, 2012). La remoción de los microcontaminantes se da por procesos abióticos como la sorción, fotólisis o degradación hidrolítica; o por procesos de biotransformación o biodegradación (Bergersen et al., 2012; Radjenović et al., 2009).

La sorción de microcontaminantes puede ser predicha con base los valores de K_d, donde los compuestos no iónicos tienden a ser absorbidos en la fracción lipídica o sorbidos en la fracción orgánica (Carballa et al., 2008; Ternes et al., 2004). Sin embargo, interacciones electrostáticas específicas son de gran importancia para ciertos fármacos, por ejemplo, los fluorochinolones tienen una K_d alta pero un K_{ow} extremadamente bajo (Ternes et al., 2004). La K_{ow} describe la hidrofobicidad de los compuestos, no está creada para discriminar entre las características de sorción de varios compuestos polares en soluciones acuosas, así, para la mayoría de los fármacos no se esperaría que la K_d pueda ser predicha en función de la K_{ow} (Ternes et al., 2004). Sin embargo, para algunas fragancias hidrofóbicas se podrían lograr resultados comparables (Carballa et al., 2008; Ternes et al., 2004).

Respecto a la sorción de microcontaminantes en los LS, existe una diferencia entre la sorción en lodo primario y lodo secundario, esta depende principalmente de los mecanismos de absorción, interacción de los grupos alifáticos y aromáticos de un compuesto con la membrada lipofílica de la célula de un microorganismo y la fracción lipídica del lodo, y de la interacción electrostática de los grupos cargado positivamente de los compuestos con la superficie cargada positivamente de los microorganismos (Ternes et al., 2004). La tendencia es que los lodos primarios estén más contaminados con microcontaminantes orgánicos, especialmente de aquellos que son hidrofóbicos (Ak et al., 2013; Chawla et al., 2014).

Respecto al efecto de la DAC y DAA sobre los microcontaminantes, la Figura 3 muestra los objetivos de estudio de las investigaciones, donde el 39% de las investigaciones ha evaluado la remoción de los microcontaminantes durante la digestión anaerobia. Un 11% de las investigaciones ha evaluado el efecto de la aplicación de un pre-tratamiento, particularmente el uso de ozono, ultrasonido, temperatura y microondas. En la Tabla 3 muestra el comportamiento de los 10 microcontaminantes determinados durante el proceso de DAC y DAA, así como la remoción reportada en la literatura y la obtenida en esta investigación.

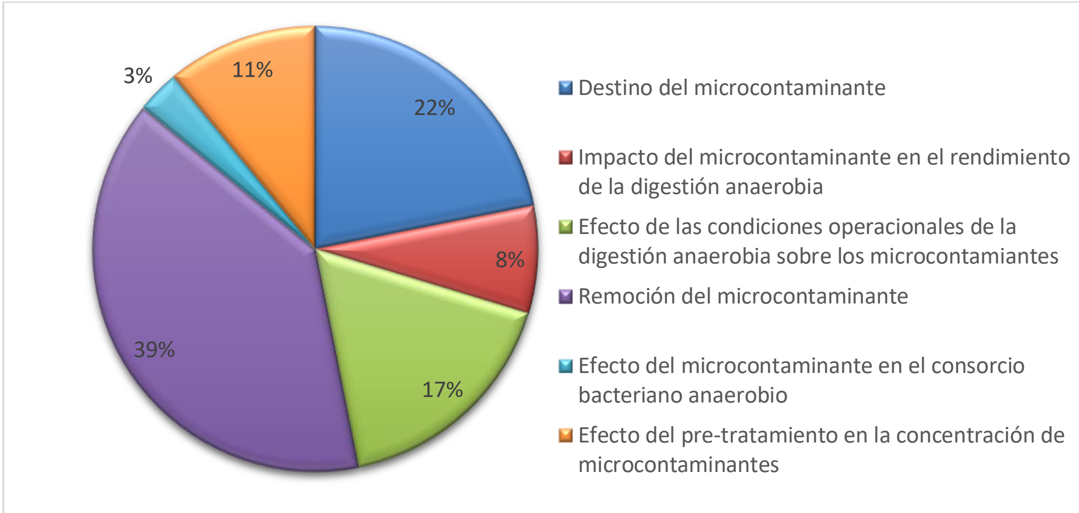

Figura 3. Objetivos de estudio de las investigaciones relacionadas a digestión anaerobia y microcontaminantes.

Entre los microcontaminantes determinados en los LS y BS se encuentran compuestos pertenecientes a diferentes grupos de interés como las fragancias, antibacteriales, antioxidantes, surfactantes y hormonas sintéticas. Se detectaron 9 de los 10 compuestos analizados, siendo el 17β-etinil estradiol, el compuesto no detectado en ninguna de las muestras de BS ni en el LS parental. En el Capítulo V se discute a profundidad el efecto de cada etapa de pre-tratamiento sobre las concentraciones de los microcontaminantes.

Durante la aplicación del pre-tratamiento secuencial aumentó la concentración de microcontaminantes entre un 13-991%, esto se relacionó principalmente con la evaporación de agua, así como al efecto extracción y recuperación producido por la etapa de ultrasonido. El proceso de DAC causó aumentos en la concentración de microcontaminantes de hasta un 360%, y el único compuesto removido en este proceso fue el triclosán en un 10%. Por otra parte, el proceso de DAA causó aumentos de hasta un 274%, y logró la remoción de tonalide y de triclosán en un 20% y 76%, respectivamente. Esta acumulación de microcontaminantes podría estar relacionada con su baja biodegradabilidad en comparación a la mayoría de los compuestos orgánicos presentes en los LS.

Al comparar el comportamiento de microcontaminantes entre los sistemas de DAC y DAA. El proceso de DAA causó una reducción de 7 de los 9 compuestos: un 73% de triclosán, 59% de tonalide, 41% de 4-nonilfenol, 19% de hidroxitolueno butilado, 31% de fenantreno, 26% de galaxolide y un 21% de pireno; y un aumento en la concentración de bisfenol A un 131% y de tert-octilfenol un 25%. Este aumento en la reducción de microcontaminantes en la DAA en comparación con la DAC, se puede asociar al efecto del ultrasonido que produce cambios físicos y químicos debido a la implosión y cavitación de las microburbujas, las cuales degradan moléculas complejas al desintegrarlas, ya sea por la reacción con los radicales hidroxilo o por una combinación de todos los fenómenos descritos (Chawla et al., 2014).

Entre los compuestos que han reportado una reducción durante la DAC están: paroxetina (98%), naproxeno (80%), sulfamethoxazole (80%) y roxitromicina (80%) (Bergersen et al., 2012; Carballa et al., 2006; McAvoy et al., 2015). Otros como fluoxetina (reducción 32%), fluvoxamina (53%), sertralina (38%), diazepam (50%) e ibuprofeno (40%) podrían tener un mayor potencial de acumulación (Bergersen et al., 2012; Carballa et al., 2006; Malmborg and Magnér, 2015). En la Tabla 3 se muestras las remociones durante la DAC y DAA, donde se reportan remociones de algunos antibióticos, estrógenos naturales, fragancias y naproxenos (Bergersen et al., 2012). Sin embargo, también se reportan algunas discrepancias respecto al efecto de la digestión anaerobia sobre los nonilfenoles y compuestos estrogénicos (Carballa et al., 2008; Chawla et al., 2014; Holbrook et al., 2002; Samaras et al., 2014). Este comportamiento se ha asociado a que compuestos lipofílicos tienen una degradación más lenta debido a la presencia de materia orgánica natural en los LS (Samaras et al., 2014). En el caso particular de los nonilfenoles, un aumento en la concentración de estos después de la estabilización de los LS se relaciona con el hecho de que son productos de degradación de otros compuestos (Samaras et al., 2014).

Considerando el efecto que la presencia de ciertos microcontaminantes pueda causar en el consorcio bacteriano se ha reportado una correlación entre el aumento de diclofenaco y triclosán y la reducción del rendimiento de la generación de metano (Symsaris et al., 2015). Por otra parte, se ha reportado que presencia de microcontaminantes tiene un efecto positivo en la producción de biogás debido al aporte de carbono de los fármacos (en particular el aporte carbonácea de los compuestos excipientes), en este caso de inhibidores de la recaptación de serotonina como: citalopram, sertralina, paroxetina, fluvoxamina y fluoxetina (Bergersen et al., 2012).

Tabla 3. Comportamiento de los contaminantes emergentes durante la digestión anaerobia convencional y avanzada.

Compuesto	Aplicación	Log K_{ow}	Log K_{oc}	Remoción (%)			
				Literatura		Este estudio	
				DAC	DAA	DAC	DAA
Tonalide	Fragancias	6.35	4.72	35-82	32-85	(-93)	20
Galaxolide		6.26	4.10	35-75	65-87	(-70)	(-26)
Triclosan	Antibacterial/antifúngico	4.66	3.93	7-81	sd	10	76
Hidroxitolueno butilado	Antioxidante usado en comida, cosméticos e industria farmacéutica	5.03	3.91	sd	sd	(-360)	(-274)
Fenantreno	Generación de energía	4.35	3.87	sd	sd	(-85)	(-27)
Pireno		4.93	4.24	sd	sd	(-71)	(-36)
4-nonilfenol	Precursor de surfactante no iónico y producto de degradación	5.99	4.28	11-91	sd	(-83)	(-8)
Tert-octilfenol	Material parental de producción de surfactantes no iónicos	5.28	4.01	sd	sd	(-194)	(-266)
Bisfenol A	Manufactura de plásticos y resinas epóxicas	3.32	3.10	78-90	sd	(-32)	(-205)
17β-etinil estradiol	Hormona sintética	4.12	2.71	(-185)-56	0-74	<LD	<LD

sd: no se encontraron datos en la bibliografía; DAC: digestión anaerobia convencional, DAA: digestión anaerobia avanzada
Referencias: (Ak et al., 2013; Carballa et al., 2006; Marta Carballa et al., 2007; Chawla et al., 2014; Hamid and Eskicioglu, 2013; Holbrook et al., 2002; Malmborg and Magnér, 2015; Ogunyoku and Young, 2014; Papa et al., 2015; Reyes-Contreras et al., 2018; Samaras et al., 2014; US EPA, 2012)

3. Efecto del pre-tratamiento en la digestión anaerobia sobre la fitotoxicidad directa e indirecta del biosólido

El tercer objetivo establecido en la tesis fue evaluar la fitotoxicidad directa e indirecta de los biosólidos producto de un proceso de digestión DAC y DAA. La fitotoxicidad indirecta se discutió en el capítulo VI. Esta se evaluó mediante la extracción de elutriado de las muestras de BS y del LS y posteriormente semillas de *Lactuca sativa*, *Raphanus sativus*, y *Triticum aestivum*, fueron expuestas a concentraciones entre 0.5 a 100% de elutriado. Por otra parte, la fitotoxicidad directa fue discutida en el capítulo VII. En este caso utilizando la matriz completa de los BS y del LS y suelo artificial como control.

Los bioensayos de fitotoxicidad son una herramienta rápida y simple para la evaluación de los efectos ecotoxicológicos de residuos que serán aplicados como remediadores de suelo (Gerber et al., 2017), además el uso de plantas se relaciona con su rol ecológico como productores primarios (Oleszczuk, 2008b;

Wilke et al., 2008). También es importante considerar que la fitotoxicidad directa representa de manera más cercana las condiciones reales de aplicación de BS y se evitan problemas de extracción, pero los altos contenidos de materia orgánica podría enmascarar el efecto de algunos compuestos, por ello la importancia de considerar también la fitotoxicidad indirecta, particularmente el elutriado que corresponde a la fracción de compuestos más biodisponibles (Alvarenga et al. 2016; Kapanen et al. 2013; Ramírez et al. 2008b).

Respecto a las plantas seleccionadas, estas han sido ampliamente utilizadas como indicadores de fitotoxicidad (Fuentes et al., 2004; Kinney et al., 2012; Oleszczuk, 2010; Oleszczuk et al., 2011; W. A. Ramírez et al., 2008) y son parte de las plantas recomendadas en diversas metodologías (OECD/OCDE, 2006). En la Tabla 4 se muestran algunos de los requerimientos nutricionales de las tres plantas utilizadas en los bioensayos. Las tres plantas poseen requerimientos nutricionales en tasas similares, las principales diferencias están en las temperaturas óptimas de germinación y en el pH óptimo; dónde *L. sativa* muestra una sensibilidad mayor en estos parámetros. Además, se reporta que *L. sativa* y *R. sativus* son poco tolerables a la salinidad, donde conductividades eléctricas mayores a 1.3 dS/m generan pérdidas en la productividad de dichos cultivos (Medina Jiménez, 2010).

Tabla 4. Principales requerimientos nutricionales de las *Lactuca sativa*, *Raphanus sativus*, y *Triticum aestivum*

Parámetro	*Lactuca sativa*	*Raphanus sativus*	*Triticum aestivum*
Temperatura óptima de germinación (°C)	15-18	20-25	20-25
pH óptimo de germinación	6.0-6.8	5.5-6.8	5.8-6.2
Nitrógeno (kg/ha)	80-190	80-100	70-200
Fósforo (kg/ha)	50-150	30-40	13-35
Potasio (kg/ha)	80-275	90-113	48-65

Fuente: Campillo (2015); Kehr et al. (2014); Medina Jiménez (2010)

En la Figura 4 se presenta la inhibición de germinación y de crecimiento resultado de los bioensayos de fitotoxicidad directa e indirecta. Tanto en los bioensayos con fitotoxicidad directa como indirecta, la mayoría de las plantas expuestas a LS mostraron inhibición en su desarrollo.

El elutriado de los BS tiene un efecto positivo en la germinación y el crecimiento de la raíz de *R. sativus* y de *T. aestivum*, con aumentos en la germinación entre 15% y 30%, y aumentos en el crecimiento de la raíz entre 38% y 54%. Mientras que para *L. sativa* todos los elutriados causaron un efecto inhibitorio con en la germinación y crecimiento de la raíz, con inhibiciones de hasta un 58%. En el caso de la fitotoxicidad directa, aplicaciones de BS sobre el 10% produjeron inhibiciones de germinación para *R. sativus*, *T. aestivum* y *L.*

sativa, entre 53-87%, 47-100%, y 55-100%, respectivamente. Por otra parte, dosis de aplicación menores al 10% presentan efectos fluctuantes en la germinación con inhibiciones variando entre 3 a 47%, (-38) a (-28)% y 0 a 40%, para *R. sativus*, *T. aestivum* y *L. sativa*, respectivamente. En la sección 4 de la discusión se profundiza sobre la relación de las características fisicoquímicas de los BS y los valores de fitotoxicidad obtenidos.

L. sativa es la plantan con mayor sensibilidad en el elutriado, tanto en la inhibición de germinación como del crecimiento de la raíz. La sensibilidad de esta planta ha sido reportada por diversas investigaciones (Alvarenga et al., 2016; Banks and Schultz, 2005). Estos resultados concuerdan con lo reportado en la literatura, de que las semillas de tubérculos, cereales y legumbres, tales como *R. sativus* y *T. aestivum*, contienen mayores reservas de alimento que las semillas de plantas herbáceas, por lo que tienen a poseer menor sensibilidad a la toxicidad (Tiquia et al., 1996).

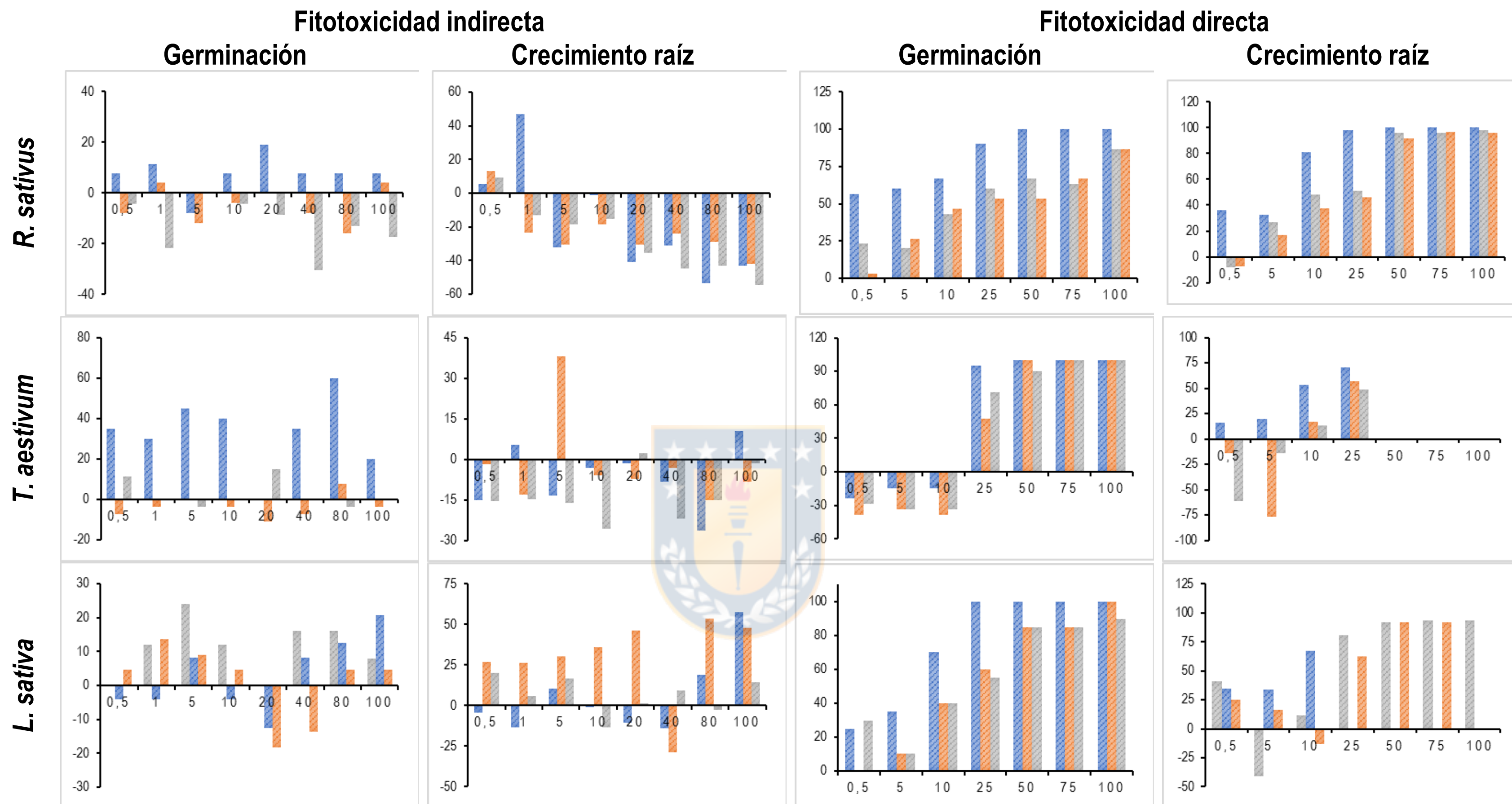

Figura 4. Comparación del porcentaje de inhibición de germinación y crecimiento de la raíz de los biosólidos de acuerdo con su fitotoxicidad directa o indirecta. Lodo sanitario: azul; biosólido de digestión anaerobia convencional: anaranjado; biosólido de digestión anaerobia avanzada: gris. Eje de x corresponde a las concentraciones en porcentaje y el eje y al porcentaje de inhibición.

En la Figura 5, se muestra una comparativa de los índices de germinación (IG). El cálculo de este se describe y discute en los capítulos VI y VII. El IG combina la germinación y el crecimiento de la raíz, entregando una estimación de la toxicidad más completa, lo que la convierte en un parámetro de toxicidad más práctico. Considerando la clasificación toxicológica dada por Roig et al. (2012), el IG se puede clasificar de la siguiente manera: efecto benéfico $IG \geq 100$, sin presencia de sustancias fitotóxicas $100 > IG \geq 80$, toxicidad moderada $80 > IG \geq 50$ y alta toxicidad $IG \leq 50$.

La fitotoxicidad indirecta de los BS indica la no presencia de sustancias fitotóxicas para *R. sativus*, efectos benéficos para *T. aestivum*. En particular, para los BS de DAA mostró efectos benéficos en el desarrollo de las plantas evaluadas. Para el caso de la fitotoxicidad directa, dosis de aplicación de BS mayores a 10% muestran efectos negativos en el desarrollo de las plantas testeadas. Sin embargo, dosis de aplicación menores o iguales al 10% indican un efecto favorable en las plantas, donde ambos BS produjeron efectos similares. Estos resultados concuerdan con los reportados en la literatura y como se discute en los capítulos VI y VII, esto se relaciona con el grado de estabilidad de la materia orgánica de los BS, así como a la degradación parcial de algunos contaminantes y a la modificación de la biodisponibilidad de estos. La aplicación del pre-tratamiento secuencial aumenta la solubilización de los LS, promueve una mayor estabilización de los lodos durante la digestión anaerobia, lo que contribuye a una menor fitotoxicidad. En la sección 4 de la discusión se profundiza sobre la relación de las características fisicoquímicas de los BS y la fitotoxicidad.

La tasa de aplicación de BS para usos agrícolas suele variar entre 5-90 ton/ha, que dependiendo de la densidad del suelo y la profundidad de aplicación correspondería aproximadamente a tasas de aplicación de 2-35 g/kg. Valores mucho menores a los arrojados en esta investigación para la EC_{50} de la germinación, crecimiento de raíz y crecimiento del brote, cuando se aplican BS provenientes de DAC y DAA (164-230 g/kg, 102-207 g/kg y 91-222 g/kg, respectivamente). En la literatura se reportan valores de EC_{50} para la germinación (21-82 g/kg) y para el crecimiento del brote (23-80 g/kg) más tóxicos que los obtenidos en esta investigación (Ramírez et al., 2008). Lo que indica que a tasas de aplicación para usos agrícolas la aplicación de los BS de DAC y DAA estudiados producen efectos benéficos en el desarrollo temprano de las plantas evaluadas o bien los BS se logran categorizar como sin presencia de sustancias fitotóxicas.

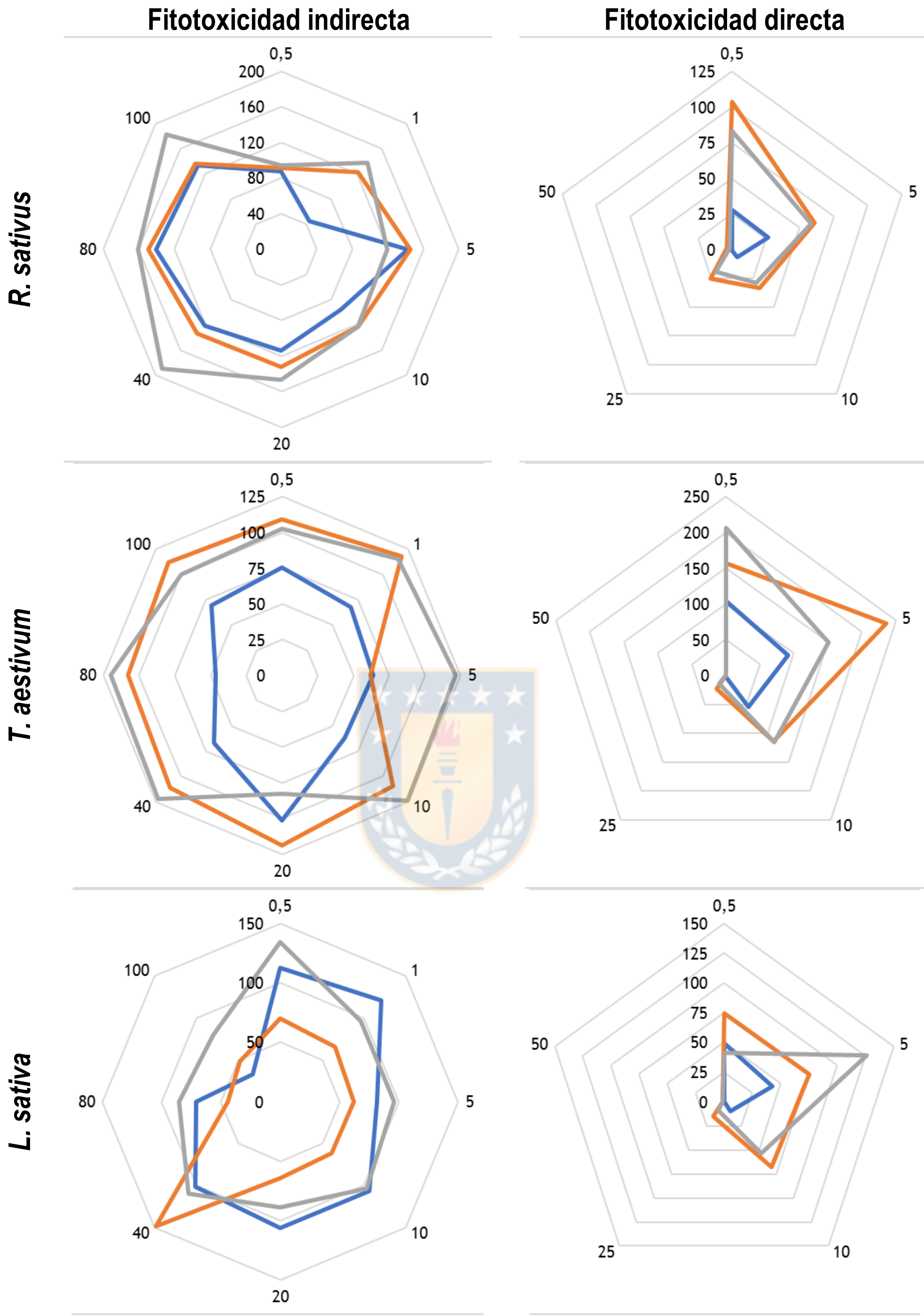

Figura 5. Comparación del Índice de Germinación de los biosólidos de acuerdo con su fitotoxicidad directa o indirecta. Lodo sanitario: azul; biosólidos de digestión anaerobia convencional: anaranjado; biosólido de digestión anaerobia avanzada: gris. Los vértices corresponden a las concentraciones utilizadas en porcentaje.

4. Relación de parámetros operacionales, características fisicoquímicas y la fitotoxicidad de los biosólidos

Integrando los resultados y discusión de las secciones anteriores como lo presentado en los capítulos del IV al VIII y para para poder responder de mejor forma al objetivo general de este trabajo se presenta en la siguiente sección donde se expone la relación de las condiciones operacionales, de las características fisicoquímicas y de los ensayos de fitotoxicidad.

La aplicación de un pre-tratamiento durante de la estabilización de LS con digestión anaerobia permite mejoras en el desempeño del reactor, aumentando la generación de biogás y la reducción de sólidos (Neumann et al., 2016). Particularmente, el pre-tratamiento secuencial aplicado en esta investigación permitió aumentos de 30% en la generación de metano y reducciones de sólidos de hasta un 33%. También, la aplicación del pre-tratamiento permitió una reducción de entre 19-73% en la concentración de microcontaminantes en el BS de DAA en comparación con el BS de DAC. Sin embargo, al comparar la concentración de microcontaminantes de los BS con el LS parental se evidenció un aumento entre 31-360% para DAC y entre 8-274% DAA lo que corresponde a concentraciones entre 0.29-59.41 µg/g y 0.12-4.99 µg/g, respectivamente.

Respecto a la fitotoxicidad, esta es resultado de la combinación de varios factores como la exposición a metales pesados, compuestos nitrogenados, ácidos grasos de bajo peso molecular y microcontaminantes (Mañas and De las Heras, 2018). No obstante, las investigaciones que relaciones la fitotoxicidad con la presencia de microcontaminantes es escasa. Entre ellas, las investigaciones de Peña et al. (2014) y Richter et al. (2016) concuerdan que la aplicación de BS en suelo reduce la fitotoxicidad de microcontaminantes como el ketoconazol, BBDA (cloruro de benzododecinio) y triclosán. Esta reducción en la fitotoxicidad debido a la aplicación de BS puede estar relacionada con el secuestro de los microcontaminantes, lo que reduciría su biodisponibilidad (Richter et al., 2016; Wu et al., 2009).

Como comparación de las concentraciones de microcontaminantes utilizadas por Peña et al. (2014) y Richter et al. (2016) para ketoconazole y triclosán fueron de 62.5-1000.0 µg/g y 7.6-46.6 µg/g, respectivamente. Mientras las concentraciones reportadas en este estudio para ambos BS fueron menores, variando entre 0.12-59.41 µg/g para los 10 compuestos cuantificados, y concentraciones entre 2.47-9.28 µg/g para el triclosán. Por tanto, considerando las concentraciones menores a las reportadas en la literatura y al posible efecto de secuestro de los microcontaminantes en la materia orgánica estabilizada, la fitotoxicidad debida a estos podría considerarse despreciable.

Respecto al contenido de metales, estos se encuentran por debajo de los límites establecidos en la normativa chilena, el DS 04, para disposición de lodos sanitarios (MINSEGPRES, 2009) (Capítulo II, Tabla 7). En la literatura se reportan concentraciones de metales mucho mayores a las encontradas en los BS estudiados (Tabla 1) y la fitotoxicidad ha sido atribuida a la presencia de compuestos nitrogenados y no a la de metales (Fuentes et al., 2004; Wollan et al., 1978). Además, dada la baja extractabilidad, es decir, poca disponibilidad de los metales en los BS, reportada por Fuentes et al. (2006) y considerando que para que el metal produzca un efecto fitotóxico este debe estar disponible para moverse del suelo a la raíz, es probable que la fitotoxicidad directa e indirecta evidenciada en esta investigación no sea respuesta directa al contenido de metales en los BS.

En el proceso de digestión anaerobia debido a la ausencia de un aceptor adecuado de electrones (oxígeno) impide la oxidación del nitrógeno, por ello las principales formas nitrogenadas encontradas en los digestatos, los BS en esta investigación, son: el nitrógeno orgánico y el nitrógeno amoniacal (Yang et al., 2018). La aplicación de un pre-tratamiento aumenta la solubilización de la materia orgánica, incluyendo los compuestos nitrogenados, esto se refleja en un aumento en la concentración de $N\text{-}NH_4^+$ un 29% mayor en los BS de DAA en comparación a los de DAC.

Ramírez et al. (2008) realizaron bioensayos con *Lolium perenne L., Brassica rapa L.* y *Trifolium pratense L* utilizando 3 extractos (agua, metanol y diclorometano con concentraciones entre 0-1500 g/L) de LS bajo tres condiciones de estabilización (DAC, DAC + compostado con chips de madera y DAC + secado térmico). El IG se correlacionó positivamente con el grado de estabilidad de la materia orgánica del BS y una correlación negativa con el contenido de nitrógeno total y amonio. Los IG para los biosólidos de DAC, DAC + compostado con chips de madera y DAC + secado térmico fueron de 2.9-45.8; 40.9-86.2 y 24.6-64.4 respectivamente. Además, los mayores efectos fitotóxicos se evidenciaron en los extractos con agua y los menores para los extractos con diclorometano, es decir, los mayores efectos se debieron principalmente a los compuestos hidrofílicos o sustancias de mediana polaridad.

Considerando lo discutido anteriormente se tiene que dosis de aplicación de BS entre 5-100 g/kg mostró efectos benéficos (IG ≥ 100) o la no presencia de sustancias fitotóxicas (100 > IG ≥ 80). El contenido de $N\text{-}NH_4^+$ sería de 87-1750 mg/kg para BS de DAC y entre 113-2260 mg/kg para los provenientes de DAA, similar a lo reportado por Tiquia (2010) donde concentraciones de $N\text{-}NH_4^+$ bajo de los 2000 mg/kg se asociaron a IG sobre 80.

Dosis de aplicación de BS de 100 g/kg equivalen aproximadamente a 26 ton/ha, en la Tabla 5. Se presentan los aportes de nutrientes asociados esta tasa de aplicación y a una tasa de 90 ton/ha que es el máximo regulado. Durante el primer año de aplicación de BS se estima que el 35% del nitrógeno total, 40% del fósforo total y 100% del potasio se encuentra disponible para que sea tomado por las plantas (Arduini et al., 2018; Sullivan et al., 2015).

Tabla 5. Aporte de nutrientes debido a la aplicación de biosólidos procedentes de digestión anaerobia convencional y avanzada.

BS	Tasa de aplicación de BS (ton/ha)	Nutrientes (ton/ha)			Nutrientes disponibles en el primer año de aplicación (kg/ha)		
		N	P	K	N	P	K
DAC	26	1.1	0.3	0.03	385	120	30
	90	3.8	1.2	0.1	1330	480	100
DAA	26	1.1	0.6	0.04	385	240	40
	90	3.8	2.1	0.2	1330	840	200

BS: biosólido; DAC: digestión anaerobia convencional; DAA: digestión anaerobia avanzada; N: nitrógeno; P: fósforo; K: potasio

5. Implicaciones de la aplicación de biosólidos provenientes de digestión anaerobia convencional y avanzada

5.1 Composición de los biosólidos y su repercusión en la aplicación en suelo

La aplicación de biosólidos en suelos es una alternativa para el reciclaje de materia orgánica, macro y micronutrientes, y al mismo tiempo reducir el uso de fertilizantes artificiales (Hosseini Koupaie and Eskicioglu, 2015). Alrededor de un 20% de la materia orgánica del biosólido corresponde a ácidos fúlvicos y húmicos (Yuning Yang et al., 2014). La materia húmica influye en las propiedades físicas, químicas y biológicas del suelo como la porosidad y estructura (rompimiento de arcillas y aumento de la resistencia a la erosión), la infiltración de agua, la humedad y la actividad biológica de los organismos del suelo, así como la dinámica de nutrientes, el crecimiento y nutrición de las plantas (Antilén et al., 2014; Nardi et al., 2009; Peña-Méndez et al., 2005; Pinton et al., 2009).

Pocos estudios han considerado el efecto de la DAA en las sustancias húmicas de los BS. Zhen et al. (2014) evaluaron la humificación de los BS después de DAA con un pre-tratamiento electro-alcalino, encontrando que los índices de humificación correspondían a materia húmica madura. Yuning Yang et al. (2014) encontraron que DAA con un pre-tratamiento térmico reducía el tamaño promedio de la materia húmica, pero aumentaba la madures de los ácidos húmicos y fúlvicos.

La cantidad de nutrientes en los BS los ha hecho atractivos para uso agrícola o recuperación de suelos, de modo que esta práctica está aumentando alrededor del mundo (Li et al., 2015; Rastetter and Gerhardt, 2015; Symsaris et al., 2015; Verlicchi and Zambello, 2015; Zhang et al., 2015). En Canadá aproximadamente 388 700 toneladas BS son producidas anualmente y cerca del 43% son aplicadas en suelos, en Israel cerca de la mitad del BS producido en Shafdan (la PTAS más grande) es utilizado en la agricultura, en Estados Unidos más del 50% de BS es aplicado en terrenos y en Europa cerca del 53% de BS son reusados en la agricultura (EPA, 2016; Hamid and Eskicioglu, 2013; Shargil et al., 2015; Stasinakis, 2012; Verlicchi and Zambello, 2015). En Chile el 39% de los BS generados son aplicados en el suelo; en la Región del Biobío un 73% de los BS generados en la Región (equivalente a un 8.72% de los generados en el país) son aplicados en el suelo, principalmente en predios forestales (SISS, 2018).

Al utilizar la digestión anaerobia para estabilizar los LS algunos países han utilizado el biogás generado como fuente de energía. Por ejemplo, en Brasil la DA de lodos sanitarios aporta un 7% al total de biogás producido en el país, en Dinamarca 21%, en Finlandia 22%, en Francia 8%, en Alemania 7%, en Noruega 33%, en Corea del Sur 38%, en Suecia 40%, en Suiza 49%, en Holanda 20% y en el Reino Unido 11% (Bachmann et al., 2015). En Chile solamente las PTAS de La Farfana, Talagante, Gran Concepción, Temuco, Osorno y El Trebal estabilizan sus lodos sanitarios mediante DA; estas generaron 65.8 millones de metros cúbicos de biogás, el 75.4% de este se aprovechó, ya sea en las mismas plantas o en las ciudades correspondientes (SISS, 2018).

Pese a lo anterior, la carga de nutrientes también puede ser fuente de contaminación, por ejemplo, la aplicación de biosólidos podría promover la existencia de nitrificadores en el suelo, aumentando la tasa de transformación de amonio a nitrato y se podría dar la lixiviación de nitratos contaminando cuerpos de agua (Gallardo et al., 2016). También la cantidad de amonio podría tener un efecto negativo en las plantas debido a una inhibición en la germinación de las semillas (Fuentes et al., 2006). Tal como se ha mostrado en esta investigación, donde los resultados apuntan a que la fitotoxicidad directa e indirecta está asociada a las concentraciones de nitrógeno amoniacal.

Algunos metales son macronutrientes (K, Mg, Ca), otros micronutrientes (Mn, Fe, Co, Cu, Mo, Ni, Zn, Cr) esenciales para el crecimiento de los organismos (Thanh et al., 2016). El exceso de estos o la presencia de metales tóxicos (Cd, Pb, Hg) pueden producir enfermedades crónicas o aguadas (Clarke et al., 2016; Gola et al., 2016; Thanh et al., 2016). Entre los factores que hacen que los metales sean tóxicos esta la interferencia que los metales no esenciales producen en el organismo: sustitución de los metales esenciales en sitios activos de las enzimas, interferencia con las vías de señalización intracelular y el metabolismo del Ca, estrés oxidativo (producción excesiva de radicales libres) o interferencia con la transcripción, traducción y reparación del ADN (Clarke et al., 2016). Entre los problemas de salud (humana) relacionados con contaminación con metales están: daño hepático (Cd, Cu, Cr, Zn), daño pulmonar (Cd, Cu, Ni), daño a la piel (Cr, Cu, Ni), daño gástrico (Cu, Cr, Zn), daño a los riñones (Cd, Cu, Cr, Cu, Ni), daño neurológico (Pb), carcinomas (Cd, Cr, Cu, Ni), entre otros (Clarke et al., 2016; Gola et al., 2016).

Los contenidos de metales entre BS producidos por medio de DAC o DAA y los LS parentales tienen a permanecer en el mismo rango, tal como se discutió en el Capítulo IV y en la sección 1 de este Capítulo. Donde la estabilización de los BS influye en la extractabilidad de los metales, haciéndolos menos biodisponibles y por ende generando menores toxicidades asociadas a la concentración de metales. Al considerar contaminación por escorrentía, Clarke et al. (2016) evaluaron cuantitativamente el riesgo por metales en aguas superficiales después de la aplicación de BS (estabilizados con DAC, cal o secado térmico) en praderas, concluyendo que la concentración de metales en los BS no representaba un riesgo para la salud humana considerando la ingesta vía agua superficial (potabilizada). De manera semejante, Hosseini Koupaie & Eskicioglu (2015) realizaron un análisis probabilístico de riesgo para la salud debido al contenido de Cd (0.80 mg/kg), Cu (524.70 mg/kg) y Zn (332.02 mg/kg) en BS aplicados como fertilizante (5-100 ton/ha), cuyos resultados indican que no existe un riesgo potencial en la salud humana debido a la aplicación a largo plazo de estos BS como fertilizante.

La presencia de agentes patógenos es otra de las consideraciones para la aplicación de BS en el suelo. Si bien la concentración de estos es menor en los BS, es importante considerar medidas para evitar el recrecimiento de las poblaciones y para prevenir el contacto con personas y animales. Entre la generación de BS y su aplicación en suelo existe un lapso que puede favorecer el recrecimiento de los patógenos. Por ejemplo, BS provenientes de la centrifugación de lodo digerido anaeróbicamente reportó un aumento de 2-4 log de coliformes fecales y *E.coli* cuando fueron almacenadas en condiciones de 25-37°C por 24h (Navab-Daneshmand et al., 2014). Otro aspecto importante, es la sobrevivencia de los patógenos en el suelo o plantas con las que tengan contacto los BS durante su aplicación en suelo. En el suelo las bacterias, virus, quistes de protozoarios y ovas helmínticas tienen un tiempo de sobrevivencia aproximado de 2 meses, 3 meses, 2 días y 2 años respectivamente; mientras que en plantas de 1 mes, 1 mes, 2 días y 1 mes respectivamente (EPA, 2003).

En el caso de la presencia de microcontaminantes surge la preocupación de la translocación de contaminantes de BS a plantas y posteriormente de plantas a humanos (Malchi et al., 2015; Shargil et al., 2015). En esta situación, la presencia de microcontaminantes en el suelo una vez aplicados los BS dependerá de condiciones físico-químicas, tanto del suelo como del BS, factores climáticos (temperatura, humedad, radiación solar) y la manera y razón de aplicación de los BS (Al-Rajab et al., 2015; Verlicchi and Zambello, 2015; Wang and Wang, 2015). Entre compuestos reportados en suelos remediados con BS están tetrabromobisfenol A, estradiol, tetraciclina, triclosán, triclorocarban, ciprofloxacín y ofloxacín (McAvoy et al., 2015; Verlicchi and Zambello, 2015). Sin embargo, compuestos como bisfenol A, nonilfenoles, triclosán y triclorocarban pueden ser degradados en condiciones aerobias una vez aplicados los BS (Petrović and Barceló, 2004; Verlicchi and Zambello, 2015; Zhang et al., 2015). Por ejemplo, el tiempo de degradación media del triclosán pasó de 80 días en el BS a 17 días cuando fue aplicado en suelo, el triclorocarban pasó de 157 a 80 días y el naproxeno pasó de 10 a 3 días (Al-Rajab et al., 2015).

Respecto a la translocación de microcontaminantes, Zhang et al. (2015) reporta concentraciones bisfenol A entre 3.12–75.5 µg/kg en hojas y entre 1.28–31.0 µg/kg en la raíz de vegetales y entre

0.62–15.0 µg/kg en cereales. Shargil et al. (2015) reporta en hojas de lechuga concentraciones de estrona y testosterona entre 0.7-6.7 y 31-73 µg/kg respectivamente. El riesgo para la salud humana de la presencia de estos compuestos en plantas de interés agronómico está siendo estudiado en detalle y algunos autores ya sugieren que la ingesta de verduras regadas con aguas residuales o abonadas con BS tiene que ser controlada (Malchi et al., 2014; Paltiel et al., 2016).

5.2 Costos económicos de la aplicación de biosólidos en suelo

El análisis costo/beneficio de la aplicación de BS no formó parte de los objetivos de esta investigación. Sin embargo, en esta sección se presentan algunas aristas sobre dicho tópico. Los costos económicos relacionados con la aplicación benéfica de BS varían de país en país, dependen de la tecnología de estabilización utilizada y de las condiciones en que se vayan a disponer los BS (sólidos o semi-sólidos), así como las distancias entre los predios de disposición y la planta de tratamiento. Sin embargo, el Reporte Económico de la Comisión Europea sobre lodos/biosólidos indica que la aplicación en suelo (uso agrícola, forestal, silvicola o restauración de suelo) es la menos costosa, mientras que la disposición en relleno sanitario o la incineración implican costos económicos mayores.

Pese a que los beneficios de los BS son conocidos y su aplicación ha sido amplia a nivel mundial, cifras concretas sobre los beneficios económicos son escasas y al menos a nivel nacional de difícil acceso. En Ontario, Canadá; más del 80% de los municipios aplican sus BS en el suelo con beneficios económicos estimados en US$250/ha debido al aporte de nutrientes como nitrógeno y fósforo, que anualmente representa ahorros para el programa de BS de Ontario de aproximadamente US$5 millones (WEAO, 2009). Sullivan et al. (2015), estimaron el valor económico del aporte de nutrientes durante el primer año de aplicación de BS procedentes de DAC, considerando el precio promedio de fertilizantes inorgánicos, el cual correspondió a $48.55 por tonelada de BS y este valor no incluye los beneficios potenciales a la calidad del suelo, los cuales son difíciles de cuantificar económicamente y dependen de cada locación donde los BS vayan a ser aplicados.

CAPÍTULO IX

CONCLUSIONES Y RECOMENDACIONES

1. Conclusiones

- El desempeño de la DAA, respecto de la producción de metano fue un 30.24% mayor que en el reactor con DAC. Debido a esto, las características fisicoquímicas de fósforo, potasio, nitrógeno y amonio en los BS de DAA fueron un 85%, 40%, 5% y 29% mayores que en los BS de DAC, respectivamente. Además, la DAC alcanzó eliminaciones de 25.6%, 34.8% y 42.3% de DQO, ST y SV, respectivamente. Mientras que la DAA logró eliminaciones de 28.9%, 33.1% y 44.1%, para los mismos parámetros. La DAC logró reducciones logarítmicas para coliformes fecales, coliformes totales y colífagos somáticos de 2.03, 1.78 y 0.37; respectivamente. Mientras, que la DAA logró remociones de 2.15, 2.05 y 1.16 para los mismos parámetros.
- Respecto al efecto de la digestión anaerobia en la concentración de microcontaminantes, ambas configuraciones resultaron en la acumulación de 8 de los 10 microcontaminantes determinados. Siendo el triclosán el único compuesto que fue eliminado durante la DAC y la DAA. Sin embargo, la DAA resultó en la disminución de 7 de los 9 compuestos detectados respecto a la DAC.
- La fitotoxicidad indirecta, las muestras de LS causaron efectos fitotóxicos que variaron desde moderado (80 > GI > 50) hasta altamente fitotóxico (50 ≥ GI). Mientras que los elutriados de BS de DAC causaron efectos positivos en el desarrollo de las plantas de *R. sativus* y *T. aestivum*, pero para *L. sativa* se observó una alta toxicidad. En el caso de los elutriados de DAA, estos mostraron un efecto benéfico o la no presencia de sustancias fitotóxicas para las tres plantas testeadas. Respecto a la fitotoxicidad directa, la aplicación de LS generó efectos fitotóxicos con porcentajes de inhibición en la germinación sobre el 50% desde dosis de aplicación de 250 g/kg. Por otra parte, la aplicación de BS entre 5-100 g/kg mostró efectos benéficos o la no presencia de sustancias fitotóxicas. Los BS de DAC fueron ligeramente más benéficos que los BS de DAA, con aumentos entre 4-20% en el IG de DAC en comparación a DAA.

Con base en los resultados obtenidos, se rechaza la hipótesis planteada de que la digestión anaerobia avanzada causaba un cambio significativo en las características fisicoquímicas, microbiológicas y fitotoxicológicas del biosólido en comparación al biosólido de digestión anaerobia convencional. Aunque la aplicación del pre-tratamiento secuencial de ultrasonido seguido de un tratamiento térmico a baja temperatura (55°C) mejoró la conversión de la materia, incrementó la producción de metano, aumentó el contenido de nutrientes en el biosólido, aumentó la remoción de organismos patógenos y de microcontaminantes. Los resultados de fitotoxicidad directa e indirecta de los biosólidos procedentes de digestión anaerobia y avanzada, no mostraron diferencias significativas.

2. Recomendaciones finales

Considerando el efecto positivo del pre-tratamiento secuencial evaluado en este estudio sobre la remoción de sólidos y en las mejoras en el desempeño de la digestión anaerobia, se recomienda evaluar un escalamiento del sistema a escala piloto. Además de operar los sistemas durante periodos mayores, que consideren los cambios temporales en las características de las aguas servidas por ende en el lodo sanitario parental, lo anterior debido a los cambios estacionales y los cambios en el patrón de uso y de consumo de la población. De igual manera, se recomienda una evaluación económica de la tecnología.

Por otra parte, se recomienda evaluar el efecto de la estacionalidad en las características de los biosólidos en particular en la concentración de microcontaminantes. De igual manera se recomienda evaluar el efecto de la aplicación de biosólidos en el mediano y largo plazo en el suelo.

También, se recomienda evaluar la ecotoxicidad de los biosólidos generados considerando otros bioensayos y niveles sistémicos. Tales como bioensayos con organismos acuáticos o bien con técnicas de metagenómica que permitan evaluar la presencia de genes de resistencia a antibióticos, por ejemplo, o la aplicación de bioensayos sin dejar de lado que ciertos ensayos de genotoxicidad o el uso de ciertos bioreceptores con levaduras pierden sensibilidad con matrices como los biosólidos.

CAPÍTULO X

REFERENCIAS

INTRODUCCIÓN, MARCO TEÓRICO Y DISCUSIÓN

Adamcová, D., Vaverková, M., Břoušková, E., 2016. The toxicity of two types of sewage sludge from wastewater treatment plant for plants in Czech Republic. J. Ecol. Eng. 17, 33–37. https://doi.org/10.12911/22998993/62283

Ak, M.S., Muz, M., Komesli, O.T., Gökçay, C.F., 2013. Enhancement of bio-gas production and xenobiotics degradation during anaerobic sludge digestion by ozone treated feed sludge. Chem. Eng. J. 230, 499–505. https://doi.org/10.1016/j.cej.2013.06.113

Al-Rajab, A.J., Sabourin, L., Lapen, D.R., Topp, E., 2015. Dissipation of triclosan, triclocarban, carbamazepine and naproxen in agricultural soil following surface or sub-surface application of dewatered municipal biosolids. Sci. Total Environ. 512–513, 480–488. https://doi.org/10.1016/j.scitotenv.2015.01.075

Al Seadi, T., Lukehurtst, C., 2012. Quality management of digestate from biogas plants used as fertiliser. IEA Bioenergy.

Alvarenga, P., Mourinha, C., Farto, M., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C., 2016. Ecotoxicological assessment of the potential impact on soil porewater, surface and groundwater from the use of organic wastes as soil amendments. Ecotoxicol. Environ. Saf. 126, 102–110. https://doi.org/10.1016/j.ecoenv.2015.12.019

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774–1781. https://doi.org/10.1016/j.chemosphere.2008.04.042

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., Cunha-Queda, A.C., Duarte, E., Vallini, G., 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33, 505–513. https://doi.org/10.1016/j.envint.2006.11.006

Alvarenga, P., Palma, P., Mourinha, C., Farto, M., Dôres, J., Patanita, M., Cunha-Queda, C., Natal-da-Luz, T., Renaud, M., Sousa, J.P.P., 2017. Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. Waste Manag. 61, 582–592. https://doi.org/10.1016/j.wasman.2017.01.004

Andrés, P., Mateos, E., Tarrasón, D., Cabrera, C., Figuerola, B., 2011. Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna. Appl. Soil Ecol. 48, 236–242. https://doi.org/10.1016/j.apsoil.2011.03.001

Antilén, M., Silva, K., Acevedo, S., Amiama, F., Faúndez, M., Knicker, H., Pizarro, C., 2014. Characterization of humic acids extracted from biosolid amended soils. J. Soil Sci. Plant Nutr. 14, 1005–1020.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781. https://doi.org/10.1016/j.pecs.2008.06.002

Appels, L., Van Assche, A., Willems, K., Degrève, J., Van Impe, J., Dewil, R., 2011. Peracetic acid oxidation as an alternative pre-treatment for the anaerobic digestion of waste activated sludge. Bioresour. Technol. 102, 4124–30. https://doi.org/10.1016/j.biortech.2010.12.070

Arata, P., Garcés, J.A., Sepúlveda, N., 2015. Evaluación de la calidad del lodoproveniente de la planta de tratamiento de aguas residuales ElTrebal y su uso agrícola, in: XXIX Congreso Interamericano de Ingeniería Sanitaria y Ambiental (AIDIS). AIDIS, Managua, Nicaragua, p. 9.

Arduini, I., Cardelli, R., Pampana, S., 2018. Biosolids affect the growth, nitrogen accumulation and nitrogen leaching of barley. Plant, Soil Environ. 64, 95–101. https://doi.org/10.17221/745/2017-PSE

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. https://doi.org/10.1016/j.apenergy.2014.02.035

Artola-Garicano, E., Borkent, I., Damen, K., Jager, T., Vaes, W.H.J., 2003. Sorption Kinetics and Microbial Biodegradation Activity of Hydrophobic Chemicals in Sewage Sludge: Model and Measurements Based on Free Concentrations. Environ. Sci. Technol. 37, 116–122. https://doi.org/10.1021/es020115y

Artuso, N., Kennedy, T.F., Connery, J., Grant, J., Schmidt, O., 2011. Effects of Biosolids at Varying Rates on Earthworms (Eisenia fetida) and Springtails (Folsomia candida). Appl. Environ. Soil Sci. 2011, 1–10. https://doi.org/10.1155/2011/519485

Bachmann, N., Jasen, J. la cour, Bochmann, G., Montpart, N., 2015. Sustainable biogas production in municipal wastewater treatment plants. IEA Bioenergy.

Banks, M.K., Schultz, K.E., 2005. Comparison of Plants for Germination Toxicity Tests in Petroleum-Contaminated Soils. Water. Air. Soil Pollut. 167, 211–219. https://doi.org/10.1007/s11270-005-8553-4

Bergersen, O., Hanssen, K.Ø., Vasskog, T., 2012. Anaerobic treatment of sewage sludge containing selective serotonin reuptake inhibitors. Bioresour. Technol. 117, 325–332. https://doi.org/10.1016/j.biortech.2012.04.086

Bonomo, M.M., Morozesk, M., Duarte, I.D., Rocha, L.D., Fernandes, M.N., Matsumoto, S.T., 2016. Sewage sludge hazardous assessment: chemical evaluation and cytological effects in CHO-k1 cells. Environ. Sci. Pollut. Res. 23, 11069–11075. https://doi.org/10.1007/s11356-016-6201-8

Bourioug, M., Gimbert, F., Alaoui-Sehmer, L., Benbrahim, M., Badot, P.-M., Alaoui-Sossé, B., Aleya, L., 2015. Effects of sewage sludge amendment on snail growth and trace metal transfer in the soil-plant-snail food chain. Environ. Sci. Pollut. Res. 22, 17925–17936. https://doi.org/10.1007/s11356-015-5006-5

Campillo, R., 2015. Gestióndel N en la productividad y calidad del cultivo de trigo.

Cano, R., Pérez-Elvira, S.I., Fdz-Polanco, F., 2015. Energy feasibility study of sludge pretreatments: A review. Appl. Energy 149, 176–185. https://doi.org/10.1016/j.apenergy.2015.03.132

Carballa, M., Fink, G., Omil, F., Lema, J.M., Ternes, T., 2008. Determination of the solid-water distribution coefficient (Kd) for pharmaceuticals, estrogens and musk fragrances in digested sludge. Water Res. 42, 287–95. https://doi.org/10.1016/j.watres.2007.07.012

Carballa, Marta, Manterola, G., Larrea, L., Ternes, T., Omil, F., Lema, J.M.M., 2007. Influence of ozone pre-treatment on sludge anaerobic digestion: Removal of pharmaceutical and personal care products. Chemosphere 67, 1444–1452. https://doi.org/10.1016/j.chemosphere.2006.10.004

Carballa, M., Omil, F., Alder, A.C., Lema, J.M., 2006. Comparison between the conventional anaerobic digestion of sewage sludge and its combination with a chemical or thermal pre-treatment concerning the removal of pharmaceuticals and personal care products. Water Sci. Technol. 53, 109. https://doi.org/10.2166/wst.2006.241

Carballa, M., Omil, F., Lema, J.M., 2009. Influence of different pretreatments on anaerobically digested sludge characteristics: Suitability for final disposal. Water. Air. Soil Pollut. 199, 311–321. https://doi.org/10.1007/s11270-008-9880-z

Carballa, M, Omil, F., Lema, J.M., 2007. Calculation Methods to Perform Mass Balances of Micropollutants in Sewage Treatment Plants. Application to Pharmaceutical and Personal Care Products (PPCPs). Environ. Sci. Technol. 41, 884–890. https://doi.org/10.1021/es061581g

Carbonell, G., Pro, J., Gómez, N., Babín, M.M.M., Fernández, C., Alonso, E., Tarazona, J.V. V., 2009. Sewage sludge applied to agricultural soil: Ecotoxicological effects on representative soil organisms. Ecotoxicol. Environ. Saf.

72, 1309–1319. https://doi.org/10.1016/j.ecoenv.2009.01.007

Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F., 2012. The effects of substrate pre-treatment on anaerobic digestion systems: a review. Waste Manag. 32, 1634–50. https://doi.org/10.1016/j.wasman.2012.04.016

Carmen Antolín, M., Muro, I., Sánchez-Díaz, M., 2010. Sewage sludge application can induce changes in antioxidant status of nodulated alfalfa plants. Ecotoxicol. Environ. Saf. 73, 436–442. https://doi.org/10.1016/j.ecoenv.2009.08.022

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J.J., Delgenès, J.P.P., Steyer, J.P.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: a review. J. Hazard. Mater. 183, 1–15. https://doi.org/10.1016/j.jhazmat.2010.06.129

Carvajal, A., Peña, M., Pérez-Elvira, S., 2013. Autohydrolysis pretreatment of secondary sludge for anaerobic digestion. Biochem. Eng. J. 75, 21–31. https://doi.org/10.1016/j.bej.2013.03.002

Celis, J., Sandoval, M., Barra, R., 2008. Plant Response to Salmon Wastes and Sewage Sludge Used as Organic Fertilizer on Two Degraded Soils Under Greenhouse Conditions. Chil. J. Agric. Res. 68, 274–283. https://doi.org/10.4067/S0718-58392008000300007

Chawla, C., Sarkar, S., Ali, S., Rehmann, L., Nakhla, G., Ray, M.B., 2014. Anaerobic digestibility of estrogens in wastewater sludge: Effect of ultrasonic pretreatment. J. Environ. Manage. 145, 307–313. https://doi.org/10.1016/j.jenvman.2014.06.017

Christofoletti, C.A., Francisco, A., Fontanetti, C.S., 2012. Biosolid Soil Application: Toxicity Tests under Laboratory Conditions. Appl. Environ. Soil Sci. 2012, 1–9. https://doi.org/10.1155/2012/518206

Christofoletti, C.A., Pedro-Escher, J., Fontanetti, C.S., 2013. Assessment of the Genotoxicity of Two Agricultural Residues After Processing by Diplopods Using the Allium cepa Assay. Water, Air, Soil Pollut. 224, 1523. https://doi.org/10.1007/s11270-013-1523-3

Clarke, R., Peyton, D., Healy, M.G., Fenton, O., Cummins, E., 2016. A quantitative risk assessment for metals in surface water following the application of biosolids to grassland. Sci. Total Environ. 566–567, 102–112. https://doi.org/10.1016/j.scitotenv.2016.05.092

Combalbert, S., Hernandez-Raquet, G., 2010. Occurrence, fate, and biodegradation of estrogens in sewage and manure. Appl. Microbiol. Biotechnol. 86, 1671–1692. https://doi.org/10.1007/s00253-010-2547-x

Coors, A., Edwards, M., Lorenz, P., Römbke, J., Schmelz, R.M., Topp, E., Waszak, K., Wilkes, G., Lapen, D.R., 2016. Biosolids applied to agricultural land: Influence on structural and functional endpoints of soil fauna on a short- and long-term scale. Sci. Total Environ. 562, 312–326. https://doi.org/10.1016/j.scitotenv.2016.03.226

Corrêa Martins, M.N., Souza, V.V. de, Silva Souza, T. da, 2016. Cytotoxic, genotoxic and mutagenic effects of sewage sludge on Allium cepa. Chemosphere 148, 481–486. https://doi.org/10.1016/j.chemosphere.2016.01.071

Dhar, B.R., Nakhla, G., Ray, M.B., 2012. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. Waste Manag. 32, 542–549. https://doi.org/10.1016/j.wasman.2011.10.007

Dohányos, M., Zábranská, J., Kutil, J., Jenícek, P., 2004. Improvement of anaerobic digestion of sludge. Water Sci. Technol. 49, 89–96.

Domene, X., Colón, J., Uras, M.V., Izquierdo, R., Àvila, A., Alcañiz, J.M., 2010. Role of soil properties in sewage sludge toxicity to soil collembolans. Soil Biol. Biochem. 42, 1982–1990. https://doi.org/10.1016/j.soilbio.2010.07.019

Domene, X., Solà, L., Ramírez, W., Alcañiz, J.M., Andrés, P., 2011. Soil bioassays as tools for sludge compost quality assessment. Waste Manag. 31, 512–522. https://doi.org/10.1016/j.wasman.2010.10.013

Du, F., 2015. Novel sludge treatment technology enhances toxic metal removal, pathogen reduction, sludge digestibility and dewaterability. The University of Queensland.

EPA, 2016. Frequent Questions about Biosolids [WWW Document]. URL https://www.epa.gov/biosolids/frequent-questions-about-biosolids (accessed 8.29.16).

EPA, 2003. Environmental Regulations and Technology. Control of pathogens and vector attraction in sewage sludge.

Ferreiro-Domínguez, N., Rigueiro-Rodríguez, A., Mosquera-Losada, M.R.R., 2011. Response to sewage sludge fertilisation in a Quercus rubra L. silvopastoral system: Soil, plant biodiversity and tree and pasture production. Agric. Ecosyst. Environ. 141, 49–57. https://doi.org/10.1016/j.agee.2011.02.009

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M.I., Ortuño, J.F., Meseguer, V.F., 2004. Phytotoxicity and heavy metals speciation of stabilised sewage sludges. J. Hazard. Mater. 108, 161–169. https://doi.org/10.1016/j.jhazmat.2004.02.014

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M.I., Pérez-Marín, A.B., Ortuño, J.F., Meseguer, V.F., 2006. Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. Environ. Pollut. 143, 355–360. https://doi.org/10.1016/j.envpol.2005.11.035

Gallardo, F., Briceño, G., Flores, M.., Diez, M.., 2016. Recycling pulp mill sludge to volcanic soil: a column leaching study. J. soil Sci. plant Nutr. 16, 248–261. https://doi.org/10.4067/S0718-95162016005000020

Gerber, M.D., Lucia, T., Correa, L., Neto, J.E.P., Correa, É.K., 2017. Phytotoxicity of effluents from swine slaughterhouses using lettuce and cucumber seeds as bioindicators. Sci. Total Environ. 592, 86–90. https://doi.org/10.1016/j.scitotenv.2017.03.075

Gianico, A., Braguglia, C.M., Cesarini, R., Mininni, G., 2013. Reduced temperature hydrolysis at 134°C before thermophilic anaerobic digestion of waste activated sludge at increasing organic load. Bioresour. Technol. 143, 96–103. https://doi.org/10.1016/j.biortech.2013.05.069

Gola, D., Malik, A., Shaikh, Z.A., Sreekrishnan, T.R., 2016. Impact of Heavy Metal Containing Wastewater on Agricultural Soil and Produce: Relevance of Biological Treatment. Environ. Process. https://doi.org/10.1007/s40710-016-0176-9

Goss, M.J., Tubeileh, A., Goorahoo, D., 2013. A Review of the Use of Organic Amendments and the Risk to Human Health, Advances in Agronomy. Elsevier. https://doi.org/10.1016/B978-0-12-407686-0.00005-1

Gray, N.F., EBSCOhost, 2010. Water technology : an introduction for environmental scientists and engineers, 3erd editi. ed. IWA Pub.

Groth, V.A., Carvalho-Pereira, T., da Silva, E.M., Niemeyer, J.C., 2016. Ecotoxicological assessment of biosolids by microcosms. Chemosphere 161, 342–348. https://doi.org/10.1016/j.chemosphere.2016.07.029

Hamid, H., Eskicioglu, C., 2013. Effect of microwave hydrolysis on transformation of steroidal hormones during anaerobic digestion of municipal sludge cake. Water Res. 47, 4966–4977. https://doi.org/10.1016/j.watres.2013.05.042

Harris, P.W., McCabe, B.K., 2015. Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater. Appl. Energy 155, 560–575. https://doi.org/10.1016/j.apenergy.2015.06.026

Haug, R.T., Stuckey, D.C., Gosset, J., McCarty, P.L., Gossett, J., McCarty, P.L., 1978. Effect of Thermal Pretreatment on Digestibility and Dewaterability of Organic Sludges. Water Pollut. Control Fed. 50, 73–85.

Hernández, T., Chocano, C., Moreno, J.-L., García, C., 2016. Use of compost as an alternative to conventional inorganic fertilizers in intensive lettuce (Lactuca sativa L.) crops—Effects on soil and plant. Soil Tillage Res. 160, 14–22. https://doi.org/10.1016/j.still.2016.02.005

Holbrook, R.D., Novak, J.T., Grizzard, T.J., Love, N.G., 2002. Estrogen receptor agonist fate during wastewater and biosolids treatment processes: A mass balance analysis. Environ. Sci. Technol. 36, 4533–4539. https://doi.org/10.1021/es020577b

Hospido, A., Carballa, M., Moreira, M., Omil, F., Lema, J.M., Feijoo, G., 2010. Environmental assessment of anaerobically digested sludge reuse in agriculture: Potential impacts of emerging micropollutants. Water Res. 44, 3225–3233. https://doi.org/10.1016/j.watres.2010.03.004

Hosseini Koupaie, E., Eskicioglu, C., 2015. Health risk assessment of heavy metals through the consumption of food crops fertilized by biosolids: A probabilistic-based analysis. J. Hazard. Mater. 300, 855–865. https://doi.org/10.1016/j.jhazmat.2015.08.018

Huguier, P., Manier, N., Chabot, L., Bauda, P., Pandard, P., 2015. Ecotoxicological assessment of organic wastes spread on land: Towards a proposal of a suitable test battery. Ecotoxicol. Environ. Saf. 113, 103–111. https://doi.org/10.1016/j.ecoenv.2014.11.017

Kapanen, A., Itävaara, M., 2001. Ecotoxicity Tests for Compost Applications. Ecotoxicol. Environ. Saf. 49, 1–16. https://doi.org/10.1006/eesa.2000.1927

Kapanen, A., Vikman, M., Rajasärkkä, J., Virta, M., Itävaara, M., 2013. Biotests for environmental quality assessment of composted sewage sludge. Waste Manag. 33, 1451–1460. https://doi.org/10.1016/j.wasman.2013.02.022

Kehr, E., Tropa, S., Martínez-Lagos, J., 2014. Aspectos generales para el cultivo de lechuga.

Kim, J., Park, C., Kim, T.-H., Lee, M., Kim, S.S.-W., Kim, S.S.-W., Lee, J., 2003. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. J. Biosci. Bioeng. 95, 271–275. https://doi.org/10.1263/jbb.95.271

Kinney, C.A., Campbell, B.R., Thompson, R., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Hay, A.G., 2012. Earthworm bioassays and seedling emergence for monitoring toxicity, aging and bioaccumulation of anthropogenic waste indicator compounds in biosolids–amended soil. Sci. Total Environ. 433, 507–515. https://doi.org/10.1016/j.scitotenv.2012.06.097

Lakhdar, A., Slatni, T., Iannelli, M.A., Debez, A., Pietrini, F., Jedidi, N., Massacci, A., Abdelly, C., 2012. Risk of municipal solid waste compost and sewage sludge use on photosynthetic performance in common crop (Triticum durum). Acta Physiol. Plant. 34, 1017–1026. https://doi.org/10.1007/s11738-011-0898-2

Lesty, Y., 2003. La valorización de los lodos de las plantas de tratamiento de aguas servidas de Aguas Andinas, in: XV Congreso de Ingeniería Sanitaria y Ambiental AIDIS. AIDIS, Concepción, Chile, p. 9.

Levantesi, C., Beimfohr, C., Blanch, A.R., Carducci, A., Gianico, A., Lucena, F., Tomei, M.C., Mininni, G., 2015. Hygienization performances of innovative sludge treatment solutions to assure safe land spreading. Environ. Sci. Pollut. Res. Int. 22, 7237–47. https://doi.org/10.1007/s11356-014-3572-6

Li, S., Li, Y., Lu, Q., Zhu, J., Yao, Y., Bao, S., 2014. Integrated drying and incineration of wet sewage sludge in combined bubbling and circulating fluidized bed units. Waste Manag. 34, 2561–2566. https://doi.org/10.1016/j.wasman.2014.08.018

Li, Y., Zhang, Y., Zhao, Z., Sun, S., Quan, X., Zhao, H., 2015. Enhancement of sludge granulation in hydrolytic acidogenesis by denitrification. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-015-7194-9

Liu, X., Liu, W., Wang, Q., Wu, L., Luo, Y., Christie, P., 2017. Soil properties and microbial ecology of a paddy field after repeated applications of domestic and industrial sewage sludges. Environ. Sci. Pollut. Res. 24, 8619–8628. https://doi.org/10.1007/s11356-017-8543-2

Lloret, E., Pascual, J.A., Brodie, E.L., Bouskill, N.J., Insam, H., Juárez, M.F.-D.D., Goberna, M., 2016. Sewage sludge addition modifies soil microbial communities and plant performance depending on the sludge stabilization process. Appl. Soil Ecol. 101, 37–46. https://doi.org/10.1016/j.apsoil.2016.01.002

Malara, A., Oleszczuk, P., 2013. Application of a battery of biotests for the determination of leachate toxicity to bacteria and invertebrates from sewage sludge-amended soil. Environ. Sci. Pollut. Res. 20, 3435–3446. https://doi.org/10.1007/s11356-012-1268-3

Malchi, T., Maor, Y., Chefetz, B., 2015. Comments on “Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation.” Environ. Int. 82, 110–112. https://doi.org/10.1016/j.envint.2015.03.014

Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B., 2014. Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks. Environ. Sci. Technol. 48, 9325–9333. https://doi.org/10.1021/es5017894

Malmborg, J., Magnér, J., 2015. Pharmaceutical residues in sewage sludge: Effect of sanitization and anaerobic digestion. J. Environ. Manage. 153, 1–10. https://doi.org/10.1016/j.jenvman.2015.01.041

Mañas, P., De las Heras, J., 2018. Phytotoxicity test applied to sewage sludge using Lactuca sativa L. and Lepidium sativum L. seeds. Int. J. Environ. Sci. Technol. 15, 273–280. https://doi.org/10.1007/s13762-017-1386-z

Mattana, S., Petrovičová, B., Landi, L., Gelsomino, A., Cortés, P., Ortiz, O., Renella, G., 2014. Sewage sludge processing determines its impact on soil microbial community structure and function. Appl. Soil Ecol. 75, 150–161. https://doi.org/10.1016/j.apsoil.2013.11.007

McAvoy, D.C., Pittinger, C.A., Willis, A.M., 2015. Biotransformation of tetrabromobisphenol A (TBBPA) in anaerobic digester sludge, soils, and freshwater sediments. Ecotoxicol. Environ. Saf. 1–8. https://doi.org/10.1016/j.ecoenv.2015.07.009

McNamara, P.J., Lapara, T.M., Novak, P.J., 2014. The impacts of triclosan on anaerobic community structures, function, and antimicrobial resistance. Environ. Sci. Technol. 48, 7393–7400. https://doi.org/10.1021/es501388v

McNamara, P.J., Wilson, C.A., Wogen, M.T., Murthy, S.N., Novak, J.T., Novak, P.J., 2012. The effect of thermal hydrolysis pretreatment on the anaerobic degradation of nonylphenol and short-chain nonylphenol ethoxylates in digested biosolids. Water Res. 46, 2937–2946. https://doi.org/10.1016/j.watres.2012.03.015

Medina Jiménez, F., 2010. Necesidades nutricionales y de riego de la lechuga. Gran Canaria, España.

MINSEGPRES, 2009. D.S. 04/09 Reglamento para el manejo de lodos generados en plantas de tratamiento de aguas servidas. Biblioteca del Congreso Nacional de Chile: Ley Chile, Chile.

Mohamed, B., Fr?d?ric, G., Laurence, A.-S., Pierre-Marie, B., Badr, A.-S., Lotfi, A., 2016. Land spreading of sewage sludge in forest plantations: effects on the growth of the duckweed Lemna minor and trace metal bioaccumulation in the snail Cantareus aspersus. Environ. Sci. Pollut. Res. 23, 9891–9900. https://doi.org/10.1007/s11356-016-6222-3

Moreira, R., Sousa, J.P.P., Canhoto, C., 2008. Biological testing of a digested sewage sludge and derived composts. Bioresour. Technol. 99, 8382–8389. https://doi.org/10.1016/j.biortech.2008.02.046

Murakami, T., Suzuki, Y., Nagasawa, H., Yamamoto, T., Koseki, T., Hirose, H., Okamoto, S., 2009. Combustion characteristics of sewage sludge in an incineration plant for energy recovery. Fuel Process. Technol. 90, 778–783. https://doi.org/10.1016/j.fuproc.2009.03.003

Muz, M., Ak, M.S., Komesli, O.T., Gökçay, C.F., 2013. An ozone assisted process for treatment of EDC's in biological sludge. Chem. Eng. J. 217, 273–280. https://doi.org/10.1016/j.cej.2012.11.134

Nafez, A.H., Nikaeen, M., Kadkhodaie, S., Hatamzadeh, M., Moghim, S., 2015. Sewage sludge composting: quality assessment for agricultural application. Environ. Monit. Assess. 187, 709. https://doi.org/10.1007/s10661-015-4940-5

Nardi, S., Carletti, P., Pizzaghello, D., Muscolo, A., 2009. Biological activities of humic substances. Biophys. Process. Involv. Nat. Org. Matter Environ. Syst. 305–330. https://doi.org/10.1002/9780470494950.ch8

Navab-Daneshmand, T., Enayet, S., Gehr, R., Frigon, D., 2014. Bacterial pathogen indicators regrowth and reduced sulphur compounds' emissions during storage of electro-dewatered biosolids. Chemosphere 113, 109–115. https://doi.org/10.1016/j.chemosphere.2014.04.012

Neumann, P., Barriga, F., Álvarez, C., González, Z., Vidal, G., 2018. Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound–thermal (55 °C) pre-treatment. Bioresour. Technol. 262, 42–51. https://doi.org/10.1016/j.biortech.2018.03.057

Neumann, P., González, Z., Vidal, G., 2017. Sequential ultrasound and low-temperature thermal pretreatment: Process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. Bioresour. Technol. 234, 178–187. https://doi.org/10.1016/j.biortech.2017.03.029

Neumann, P., Pesante, S., Venegas, M., Vidal, G., 2016. Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Rev. Environ. Sci. Bio/Technology 15, 173–211. https://doi.org/10.1007/s11157-016-9396-8

Nogueirol, R., Melo, W., Alleoni, L., 2013. Testing Extractants for Cu, Fe, Mn, and Zn in Tropical Soils Treated with Sewage Sludge for 13 Consecutive Years. Water, Air, Soil Pollut. 224, 1–13. https://doi.org/10.1007/s11270-013-1557-6

OECD/OCDE, 2006. Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD. https://doi.org/10.1787/9789264070066-en

Ogunyoku, T.A., Young, T.M., 2014. Removal of Triclocarban and Triclosan during Municipal Biosolid Production. Water Environ. Res. 86, 197–203. https://doi.org/10.2175/106143013X13807328849378

Oleszczuk, P., 2010. Testing of different plants to determine influence of physico-chemical properties and contaminants content on municipal sewage sludges phytotoxicity. Environ. Toxicol. 25, 38–47. https://doi.org/10.1002/tox.20470

Oleszczuk, P., 2008a. Heterocypris incongruens as a tool to estimate sewage sludge toxicity. Environ. Toxicol. Chem. 27, 864–872. https://doi.org/10.1897/07-234.1

Oleszczuk, P., 2008b. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69, 496–505. https://doi.org/10.1016/j.ecoenv.2007.04.006

Oleszczuk, P., Hollert, H., 2011. Comparison of sewage sludge toxicity to plants and invertebrates in three different soils. Chemosphere 83, 502–509. https://doi.org/10.1016/j.chemosphere.2010.12.061

Oleszczuk, P., Jośko, I., Xing, B., 2011. The toxicity to plants of the sewage sludges containing multiwalled carbon nanotubes. J. Hazard. Mater. 186, 436–442. https://doi.org/10.1016/j.jhazmat.2010.11.028

Oleszczuk, P., Malara, A., Jośko, I., Lesiuk, A., 2012a. The phytotoxicity changes of sewage sludge-amended soils. Water, Air, Soil Pollut. 223, 4937–4948. https://doi.org/10.1007/s11270-012-1248-8

Oleszczuk, P., Rycaj, M., Lehmann, J., Cornelissen, G., 2012b. Influence of activated carbon and biochar on phytotoxicity of air-dried sewage sludges to Lepidium sativum. Ecotoxicol. Environ. Saf. 80, 321–326. https://doi.org/10.1016/j.ecoenv.2012.03.015

Oliveira, E., Barata, C., Piña, B., 2015. Endocrine Disruption in the Omics Era: New Views, New Hazards, New Approaches. Open Biotechnol. J. 9. https://doi.org/10.2174/1874070720151112E023

Paltiel, O., Fedorova, G., Tadmor, G., Kleinstern, G., Maor, Y., Chefetz, B., 2016. Human Exposure to Wastewater-Derived Pharmaceuticals in Fresh Produce: A Randomized Controlled Trial Focusing on Carbamazepine. Environ. Sci. Technol. 50, 4476–4482. https://doi.org/10.1021/acs.est.5b06256

Papa, M., Gonzalez, L., Feretti, D., Ceretti, E., Mazzoleni, G., Steimberg, N., Pedrazzani, R., Bertanza, G., Lema, J., Carballa, M., 2015. Fate and removal of organic micropollutants during anaerobic digestion of sewage sludge: are chemical + biological assays a winning combination?, in: AD 14th World Congress on Anaerobic Digestion. Viña del Mar, Chile.

Peña-Méndez, E.M., Havel, J., Patočka, J., 2005. Humic substances – compounds of still unknown structure : applications in agriculture , industry , environment , and biomedicine. J. Appl. Biomed. 3, 13–24.

Peña, A., Mingorance, M.D., Guzmán, I., Sánchez, L., Fernández-Espinosa, A.J., Valdés, B., Rossini-Oliva, S., 2014. Protecting effect of recycled urban wastes (sewage sludge and wastewater) on ryegrass against the toxicity of pesticides at high concentrations. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2014.04.002

Pérez-Elvira, S.I., Nieto Diez, P., Fdz-Polanco, F., 2006. Sludge minimisation technologies. Rev. Environ. Sci. Bio/Technology 5, 375–398. https://doi.org/10.1007/s11157-005-5728-9

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2014. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27. https://doi.org/10.1016/j.watres.2014.08.053

Petrović, M., Barceló, D., 2004. Analysis and fate of surfactants in sludge and sludge-amended soils. TrAC Trends Anal. Chem. 23, 762–771. https://doi.org/10.1016/j.trac.2004.07.015

Pinton, R., Cesco, S., Varanini, Z., 2009. Role of Humic Substances in the Rhizosphere. Biochemistry 341–366.

Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43, 831–841. https://doi.org/10.1016/j.watres.2008.11.043

Ramírez, W.A., Domene, X., Andrés, P., Alcañiz, J.M., 2008. Phytotoxic effects of sewage sludge extracts on the germination of three plant species. Ecotoxicology 17, 834–844. https://doi.org/10.1007/s10646-008-0246-5

Ramírez, W.A.A., Domene, X., Ortiz, O., Alcañiz, J.M.M., 2008. Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresour. Technol. 99, 7168–7175. https://doi.org/10.1016/j.biortech.2007.12.072

Rastetter, N., Gerhardt, A., 2015. Toxic potential of different types of sewage sludge as fertiliser in agriculture:

ecotoxicological effects on aquatic and soil indicator species. J. Soils Sediments 15, 565–577. https://doi.org/10.1007/s11368-014-1031-0

Renaud, M., Chelinho, S., Alvarenga, P., Mourinha, C., Palma, P., Sousa, J.P., Natal-da-Luz, T., 2017. Organic wastes as soil amendments – Effects assessment towards soil invertebrates. J. Hazard. Mater. 330, 149–156. https://doi.org/10.1016/j.jhazmat.2017.01.052

Reyes-Contreras, C., Neumann, P., Barriga, F., Venegas, M., Domínguez, C., Bayona, J.M., Vidal, G., 2018. Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization. Environ. Technol. 1–8. https://doi.org/10.1080/09593330.2018.1534892

Richter, E., Roller, E., Kunkel, U., Ternes, T.A., Coors, A., 2016. Phytotoxicity of wastewater-born micropollutants – Characterisation of three antimycotics and a cationic surfactant. Environ. Pollut. 208, 512–522. https://doi.org/10.1016/J.ENVPOL.2015.10.024

Roig, N., Sierra, J., Nadal, M., Martí, E., Navalón-Madrigal, P., Schuhmacher, M., Domingo, J.L., 2012. Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants. Sci. Total Environ. 425, 99–109. https://doi.org/10.1016/j.scitotenv.2012.03.018

Ros, C. Da, Cavinato, C., Micolucci, F., M.Gottaro, Cecchi, F., Bolzonella, D., 2015. Monitoring of Treviso anaerobic co-digestion plant: focus on micro-pollutants fate, in: AD 14th World Congress on Anaerobic Digestion. Viña del Mar, Chile.

Rossini-Oliva, S., Mingorance, M.D., Peña, A., 2017. Effect of two different composts on soil quality and on the growth of various plant species in a polymetallic acidic mine soil. Chemosphere 168, 183–190. https://doi.org/10.1016/j.chemosphere.2016.10.040

Şahinkaya, S., Sevimli, M.F., 2013. Sono-thermal pre-treatment of waste activated sludge before anaerobic digestion. Ultrason. Sonochem. 20, 587–594. https://doi.org/10.1016/j.ultsonch.2012.07.006

Samaras, V.G., Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Lekkas, T.D., 2014. Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. Bioresour. Technol. 162, 365–372. https://doi.org/10.1016/j.biortech.2014.03.154

Samolada, M.C., Zabaniotou, A.A., 2014. Comparative assessment of municipal sewage sludge incineration, gasification and pyrolysis for a sustainable sludge-to-energy management in Greece. Waste Manag. 34, 411–420. https://doi.org/10.1016/j.wasman.2013.11.003

Shargil, D., Gerstl, Z., Fine, P., Nitsan, I., Kurtzman, D., 2015. Impact of biosolids and wastewater effluent application to agricultural land on steroidal hormone content in lettuce plants. Sci. Total Environ. 505, 357–366. https://doi.org/10.1016/j.scitotenv.2014.09.100

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28, 347–358. https://doi.org/10.1016/j.wasman.2006.12.010

SISS, 2018. Informe de Gestión del Sector Sanitario 2017. Santiago, Chile.

Speece, R.E., 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries: Corrosion Control. Fields Publishing, Incorporated.

Spellman, F.R., 2009. Handbook of water and wastewater treatment plant operations. CRC Press/Taylor & Francis.

Stasinakis, A.S., 2012. Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour. Technol. 121, 432–440. https://doi.org/10.1016/j.biortech.2012.06.074

Sullivan, D., Cogger, C., Bary, A., 2015. Fertilizing with Biosolids.

Symsaris, E.C., Fotidis, I.A., Stasinakis, A.S., Angelidaki, I., 2015. Effects of triclosan, diclofenac, and nonylphenol on mesophilic and thermophilic methanogenic activity and on the methanogenic communities. J. Hazard. Mater. 291, 45–51. https://doi.org/10.1016/j.jhazmat.2015.03.002

Tchobanoglous, G., Burton, F., Stensel, D., 2003. Wastewater Engineering; Treatment and Reuse. McGraw-Hill, New York, EEUU.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid-water distribution coefficient (K d) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084. https://doi.org/10.1016/j.watres.2004.07.015

Thanh, P.M., Ketheesan, B., Yan, Z., Stuckey, D., 2016. Trace metal speciation and bioavailability in anaerobic digestion: A review. Biotechnol. Adv. 34, 122–136. https://doi.org/10.1016/j.biotechadv.2015.12.006

Tian, X., Wang, C., Trzcinski, A.P., Lin, L., Ng, W.J., 2015. Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment. Chemosphere 140, 63–71. https://doi.org/10.1016/j.chemosphere.2014.09.007

Tiquia, S.M., 2010. Reduction of compost phytotoxicity during the process of decomposition. Chemosphere. https://doi.org/10.1016/j.chemosphere.2010.02.040

Tiquia, S.M., Tam, N.F.Y., Hodgkiss, I.J., 1996. Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ. Pollut. 93, 249–256. https://doi.org/10.1016/S0269-7491(96)00052-8

Tiwary, A., Williams, I.D., Pant, D.C., Kishore, V.V.N., 2015. Emerging perspectives on environmental burden minimisation initiatives from anaerobic digestion technologies for community scale biomass valorisation. Renew. Sustain. Energy Rev. 42, 883–901. https://doi.org/10.1016/j.rser.2014.10.052

Traversi, D., Villa, S., Lorenzi, E., Degan, R., Gilli, G., 2012. Application of a real-time qPCR method to measure the methanogen concentration during anaerobic digestion as an indicator of biogas production capacity. J. Environ. Manage. 111, 173–177. https://doi.org/10.1016/j.jenvman.2012.07.021

Tyagi, V.K., Lo, S.-L., 2011. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review. Rev. Environ. Sci. Bio/Technology 10, 215–242. https://doi.org/10.1007/s11157-011-9244-9

US EPA, 2012. Estimation Programs Interface Suite. EPI Suite. United States Environmental Protection Agency, Washingron, DC, USA.

van Lier, J., Mahmound, N., Zeeman, G., 2008. Anaerobic Wastewater Treatment, in: Henze, M., Van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D. (Eds.), Biological Wastewater Treatment: Principles, Modelling and Design. IWA Publishing, London, UK, pp. 401–442.

Vera, I., Sáez, K., Vidal, G., 2013. Performance of 14 full-scale sewage treatment plants: Comparison between four aerobic technologies regarding effluent quality, sludge production and energy consumption. Environ. Technol. (United Kingdom) 34, 2267–2275. https://doi.org/10.1080/09593330.2013.765921

Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. Sci. Total Environ. 538, 750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108

Wang, S., Wang, H., 2015. Adsorption behavior of antibiotic in soil environment: a critical review. Front. Environ. Sci.

Eng. 9, 565–574. https://doi.org/10.1007/s11783-015-0801-2

WEAO, 2009. Frequently asked questions on biosolids managements. Ontario, Canadá.

WHO, 2018. Saneamiento [WWW Document]. World Heal. Organ. URL https://www.who.int/es/news-room/fact-sheets/detail/sanitation (accessed 4.15.19).

Wilke, B.-M., Riepert, F., Koch, C., Kühne, T., 2008. Ecotoxicological characterization of hazardous wastes. Ecotoxicol. Environ. Saf. 70, 283–293. https://doi.org/10.1016/j.ecoenv.2007.10.003

Wollan, E., Davis, R.D., Jenner, S., 1978. Effects of sewage sludge on seed germination. Environ. Pollut. https://doi.org/10.1016/0013-9327(78)90037-X

Wu, C., Spongberg, A.L., Witter, J.D., 2009. Sorption and biodegradation of selected antibiotics in biosolids. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 44, 454–461. https://doi.org/10.1080/10934520902719779

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Yang, Yuning, Li, H., Li, J., 2014. Variation in humic and fulvic acids during thermal sludge treatment assessed by size fractionation, elementary analysis, and spectroscopic methods. Front. Environ. Sci. Eng. 8, 854–862. https://doi.org/10.1007/s11783-014-0755-9

Yang, Ying, Yu, K., Xia, Y., Lau, F.T.K., Tang, D.T.W., Fung, W.C., Fang, H.H.P., Zhang, T., 2014. Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants. Appl. Microbiol. Biotechnol. 98, 5709–5718. https://doi.org/10.1007/s00253-014-5648-0

Yang, Y., Zhang, Y., Li, Y., Zhao, H., Peng, H., 2018. Nitrogen removal during anaerobic digestion of wasted activated sludge under supplementing Fe(III) compounds. Chem. Eng. J. 332, 711–716. https://doi.org/10.1016/J.CEJ.2017.09.133

Zhang, Z., Le Velly, M., Rhind, S.M., Kyle, C.E., Hough, R.L., Duff, E.I., McKenzie, C., 2015. A study on temporal trends and estimates of fate of Bisphenol A in agricultural soils after sewage sludge amendment. Sci. Total Environ. 515–516, 1–11. https://doi.org/10.1016/j.scitotenv.2015.01.053

Zoghlami, R., Mokni-Tlili, S., Hamdi, H., Naceur Khelil, M., Ben Aissa, N., Jedidi, N., 2016. Physicochemical, microbiological and ecotoxicological characterization of urban sewage sludge destined for agricultural reuse. J. New Sci. 27, 1540–1548.

ANEXO I

HITOS Y PRODUCTOS DE LA TESIS

Publicaciones en revistas indexadas

Venegas M, Leiva AM, & Vidal G. (2018). Influence of Anaerobic Digestion with Pretreatment on the Phytotoxicity of Sewage Sludge. *Water, Air, & Soil Pollution*, 229(12), 381. doi:10.1007/s11270-018-4025-5

Reyes-Contreras C, Neumann P, Barriga F, **Venegas M**, Domínguez C, Bayona JM & Vidal G. (2018). Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization. *Environmental Technology*, 1–8. doi:0.1080/09593330.2018.1534892

Venegas, M, Leiva, AM, Bay-Schmith, E, Silva, J & Vidal, G. (2019). Phytotoxicity of biosolids for soil application: Influence of conventional and advanced anaerobic digestion with sequential pre-treatment. *Environmental Technology & Innovation*, 16. https://doi.org/10.1016/j.eti.2019.100445

Venegas M., Leiva A.M., Reyes-Contreras C., Neumann P., Piña B. and Vidal G. Presence and fate of micropollutants during anaerobic digestion of sewage sludge and their implications for the circular economy: a mini-review. *Reviews in Environmental Science and Bio/Technology*. (in progress).

Presentaciones en congresos internacionales

Venegas M, Neumann P, Chamorro S, y Vidal G. 2016. Quality bioindicators for biosolids coming from anaerobic digestion with pre-treatment processes. HSM2016: 2nd IWA Conference on Holistic Sludge Management. Malmö, Suecia 7-9 Junio. Presentación Póster.

Venegas M, Barriga F, Neumann P, y Vidal G. 2016. Physical, chemical and toxicological characterization of biosolids from conventional and advanced anaerobic digestion. CRHIAM-INOVAGRI International Meeting. Concepción, Chile 24-26 Octubre. Presentación póster.

Álvarez C, **Venegas M**, Neumann P, Barriga F, y Vidal G. 2017. Caracterización de biosólidos estabilizados por digestión anaeróbica provenientes de la industria sanitaria. Water in industry. Santiago, Chile 7-9 Junio. Presentación póster.

Venegas M, Neumann P, Barriga F, y Vidal G. 2018. Evaluation of biosolids from advanced anaerobic digestion: technology improvement and sludge reuse approach. SMICE2018 Sludge Management in Circular Economy. Roma, Italia 23-25 Mayo. Presentación póster.

Venegas M, y Vidal G. Effect of pre-treatment on anaerobic digestion of the biosolids from sewage evaluated by phytotoxicity. DAAL XIII Taller y Simposio Latinoamericano de Digestión Anaerobia. Medellín, Colombia 21-24 Octubre. Presentación oral.

Presentaciones en congresos nacionales

Venegas M, Barriga F, Neumann P, y Vidal G. 2017. Fitotoxicidad indirecta de biosólidos provenientes de digestión anaerobia convencional y avanzada. XXII Congreso Chileno de Ingeniería Sanitaria y Ambiental AIDIS-Chile. Iquique, Chile 16-18 Octubre. Presentación oral.

Co-guía de tesis de pregrado

Fernández Sereño, Gabriela. 2019. Efecto del pre-tratamiento sobre la lixiviación de nitrógeno y fósforo de biosólidos provenientes de digestión anaeróbica, en un suelo franco-limoso. Tesis presentada a la Facultad de Ciencias Ambientales de la Universidad de Concepción, para optar al título de Ingeniería Ambiental.

ANEXO II

PORTADA DE LOS ARTÍCULOS PUBLICADOS

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Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization

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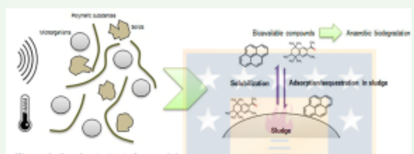
ABSTRACT

Organic micropollutants (OMP) in the household and industrial wastewater are not efficiently removed by conventional treatment processes and a significant fraction ends in sludge. Proper valorization technologies become fundamental to attain sustainable sewage sludge management, with anaerobic digestion (AD) as one of the preferred strategies. However, it exhibits some limitations that can be overcome with pre-treatment processes. In this study, the influence of different pre-treatment configurations over OMP concentration and removal during AD was assessed. The incorporation of a sequential US – TT-PT resulted in decreased concentrations of 7 of the 9 detected compounds in biosolids compared to conventional AD digestate, with bisphenol-A and ter–octylphenol showing the opposite effect. The results suggest that the assessed PT could improve the removal of sequestered or highly hydrophobic compounds through their solubilization and increased bioavailability.



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KEYWORDS
Anaerobic digestion; sewage sludge; ultrasound; thermal treatment; organic micropollutants

1. Introduction

Sewage treatment represents a fundamental mainstay for public health protection. Through successive physical, chemical and biological processes, efficient removal of solids, organic matter, nutrients and pathogens can be achieved. However, removal of organic micropollutants (OMP) that enter in the sewage system from household and industrial wastewaters is not always efficient, being influenced by the physicochemical characteristics of the pollutant and the treatment technology used [1]. Furthermore, in most conventional treatment processes the fate of a significant fraction of the different OMP is the sludge generated during the depuration process [2–4].

Sewage sludge (SS) generated during activated sludge processes is characterized by high concentrations of solids (2–12% total solids for liquid sludge and 12–40% for dehydrated sludge), organic matter (55–85% of volatile solids in dry basis), pathogens (10^9 faecal coliforms/100 mL, 2500–70,000 virus/100 mL, 200–1000 Helminth/100 mL) and nutrients (>8 mgP/kg, >30 mgN/kg, >3 mgK/kg) [5,6]. Therefore, proper treatment and disposal alternatives are necessary in order to avoid impacts such as pathogen spread, toxicological effects or uncontrolled input of nutrients to natural ecosystems. In this scenario, management of sludge can rise up to 50% of the total operational costs of current-technology depuration plants [7].

One of the preferred strategies is the use of anaerobic digestion (AD) followed by land application, which allows biogas production and nutrient re-cycling by means of digestate use as organic fertilizer. However, SS conversion during AD is limited by the low hydrolysis rate of solids and complex organic compounds, which in turn

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Influence of Anaerobic Digestion with Pretreatment on the Phytotoxicity of Sewage Sludge

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Abstract The aim of this study is to evaluate the influence of anaerobic digestion with pretreatment on the phytotoxicity of sewage sludge. The phytotoxicity was evaluated on sewage sludge (SS) and biosolids that came from conventional anaerobic digestion (CAD) and anaerobic digestion with a pretreatment by sequential ultrasound and low-thermal hydrolysis, called advance anaerobic digestion (AAD). To compare the phytotoxicity, eight elutriate concentrations (0.5–100% *v*/*v*) from SS, CAD, and AAD were studied on three testing plants: *Lactuca sativa*, *Raphanus sativus*, and *Triticum aestivum*. The percentages of seed germination inhibition, root elongation, and germination index (GI) were evaluated. GI is an phytotoxicity indicator that combines seed germination and root growth, therefore reflecting a more complete estimation of toxicity. Phytotoxicity assays showed that SS, CAD, and AAD elutriates have a beneficial effect on *R. sativus*. Similar results were observed for *T. aestivum* for CAD and AAD, with GI values up to 80% in both biosolids. Only for SS, moderate toxicity was observed in *T. aestivum*. Moreover, *L. sativa* showed GI values below 50% for SS and CAD, which reflected high toxicity. Only for AAD, no presence of phytotoxic substances was observed in *L. sativa*. This study concluded that biosolids from AAD improved the plants' development with a GI above 78% with respect to biosolids from SS and CAD and reduced the phytotoxicity of sewage biosolid.

Keywords Phytotoxicity · Biosolid · Anaerobic digestion · Pretreatment · Sewage sludge

1 Introduction

In recent decades, sewage sludge (SS) generation has increased due to the aerated technology (i.e., activated sludge) used in sewage treatment (Stiborova et al. 2017). The accumulation of non-stabilized SS generates environmental problems for populations and ecosystems (Alvarenga et al. 2016). Conventional anaerobic digestion (CAD) generates a stabilized SS (called biosolid) that can be used as a soil amendment with beneficial effects (Fuentes et al. 2004). In terms of methane production, CAD can be improved by using a pretreatment (Neumann et al. 2016). Thus, advanced anaerobic digestion (AAD) consisting of a sequential ultrasound and low-thermal hydrolysis (55–90 °C) has been proposed as an economically feasible pretreatment technology (Carvajal et al. 2013; Dhar et al. 2012; Neumann et al. 2017).

The potential risks of biosolids being used as a soil amendment are studied by ecotoxicological assessment (Walter et al. 2006). Specifically, the phytotoxicity assay is a quick tool to evaluate the ecotoxicity of biosolids due to its simplicity and sensitivity (Gerber et al. 2017). A common phytotoxicity indicator is the germination

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Phytotoxicity of biosolids for soil application: Influence of conventional and advanced anaerobic digestion with sequential pre-treatment

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ABSTRACT

The aim of this study was to evaluate the influence of different anaerobic digestion processes on the phytotoxicity of biosolids (BS) for soil application. Three different plants species, *Lactuca sativa*, *Raphanus sativus*, and *Triticum aestivum*, were subjected to seven treatments consisting of different amounts of BS (5–1000 g/kg). In this case, the biosolid samples came from the stabilization of sewage sludge (SS) and were obtained by using conventional anaerobic digestion (CAD) or advanced anaerobic digestion (AAD), which includes a sequential ultrasound pre-treatment and a low-temperature thermal process. To evaluate the phytotoxicity, the germination inhibition percentages, root and sprout growth, germination index (GI), and EC_{50} were determined. The results showed that the application of SS with a concentration of 250 g/kg inhibited the germination of *L. sativa*, *R. sativus* and *T. aestivum*, with percentages that varied between 50%–100%. This study concludes that the application of 100 g/kg BS shows either beneficial effects (GI $\geq$100) or no presence of phytotoxicity (100 > GI $\geq$ 80). These results were corroborated by EC_{50}averages of 164 g/kg and 159 g/kg obtained at endpoints from plants treated with BS after CAD and AAD, respectively.

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1. Introduction

Under the threat of climate change and the limited availability of natural resources, such as water and phosphorus, a paradigmatic shift from "end-of-pipe-approach" to "resources-oriented-approach" is necessary. Moreover, under this new perspective, the life cycle of materials has to be considered in the framework of a circular economy (Alvarenga et al., 2017; Papa et al., 2017). In this context, wastewater treatment plants (WWTPs) should be designed with technologies that allow for closing cycles and the recovery of resources (Papa et al., 2017).

Biosolids (BS) (stabilized sewage sludge (SS)) are resources generated by WWTPs that could be considered as raw materials for soil amendment use in agriculture due to their physicochemical properties (Alvarenga et al., 2017). The use of BS on soil offers economic and environmental benefits because they: (a) allow for the recycling of micro- and macronutrients (nitrogen, phosphorus, and potassium); (b) enhance soil organic carbon storage; (c) promote the formation of stable aggregates; (d) improve the water holding capacity and soil cationic exchange and aeration; and e) promote

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