Use of halophytes as biofilter to decrease organic and inorganic contaminants in water and their further use for biogas production

Von der Naturwissenschaftlichen Fakultät
der Gottfried Wilhelm Leibniz Universität Hannover
zur Erlangung des Grades

Doktor der Naturwissenschaften
Dr. rer. nat.

genehmigte Dissertation
von

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geboren am 19.12.1983 in Morazán, Guatemala

2017

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Tag der Promotion: 09.12.2016

Erklärung kumulative Dissertation:

aus:
Gemeinsame Ordnung für die Promotion zur Doktorin der Naturwissenschaften oder zum Doktor der Naturwissenschaften (Dr. rer. nat.) an der Gottfried Wilhelm Leibniz Universität Hannover (25.03.2013), § 8 Dissertation, (3):

A:
„[…][2] Es ist eine ausführliche Darstellung voranzustellen, die eine kritische Einordnung der Forschungsthemen und wichtigsten Erkenntnisse aus den Publikationen in den Kontext der wissenschaftlichen Literatur zum Thema vornimmt […]“

Die voranzustellende ausführliche Darstellung ist in dieser Arbeit aufgeteilt in die Kapitel 1 und 6.

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Publication (Chapter 2)

Turcios AE & Papenbrock J, (2014): Sustainable treatment of aquaculture effluents – what can we learn from the past for the future?, Sustainability, 6, 836-856. doi:10.3390/su6020836

The basic structure of this paper was suggested by J. Papenbrock. In all other aspects both authors contributed equally to this article.

Publication (Chapter 3)

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *Tripolium pannonicum*, Biomass & Bioenergy. 87: 69-77. doi:10.1016/j.biombioe.2016.01.013

The overall idea for the experiments was developed by A. Turcios, D. Weichgrebe, and J. Papenbrock. The more detailed design of the experiment was done by A. Turcios and J. Papenbrock. The experiments were conducted by A. Turcios as well as the data analysis. The manuscript was written by A. Turcios and J. Papenbrock, and reviewed by D. Weichgrebe.

Publication (Chapter 4)

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Potential use of the facultative halophyte *Chenopodium quinoa* Willd. as substrate for biogas production cultivated with different concentrations of sodium chloride under hydroponic conditions, Bioresource Technology, 203: 272-279. doi: 10.1016/j.biortech.2015.12.061

The overall idea for the experiments was developed by A. Turcios, D. Weichgrebe, and J. Papenbrock. The more detailed design of the experiment was done by A. Turcios and J. Papenbrock. The experiments were conducted by A. Turcios as well as the data analysis. The manuscript was written by A. Turcios and J. Papenbrock, and reviewed by D. Weichgrebe.

Publication (Chapter 5)

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Uptake and biodegradation of the antimicrobial sulfadimidine by the species *Tripolium pannonicum* acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production, Bioresource Technology, 219: 687-693. doi: 10.1016/j.biortech.2016.08.047

The overall idea and the detailed design of the experiment was developed by A. Turcios and J. Papenbrock. D. Weichgrebe provided technical support on anaerobic digestion. The experiments were conducted by A. Turcios as well as the data analysis. The manuscript was written by A. Turcios and J. Papenbrock.

Summary

This work analyses the potential use of halophytes as biofilter and their further use for biogas production through an anaerobic digestion process. In this regard, different halophytic species were tested to study the capability to grow with different salt concentrations under hydroponic conditions. Plant growth parameters were studied as well as the biofilter potential to decrease nitrates, phosphates and the antibiotic sulfadimidine (SDI) and subsequently the biomass was used as substrate for biogas production. The species Sea Aster (*Tripolium pannonicum*) was cultivated under hydroponic conditions with different salt concentrations and fresh biomass was used to determine the biogas production potential. According to the findings, it is possible to produce high yields of methane using biomass from halophytes cultivated in the presence of salt. Methane yields are positively influenced by the salt in the culture medium, with an optimal concentration between 10 $g \cdot L^{-1}$ and 30 $g \cdot L^{-1}$ NaCl. However, high concentrations of salt in the anaerobic reactors itself inhibit the biogas and methane production but using a salt-adapted inoculum increases the biogas yield in comparison to the non-adapted inoculum. The halophyte *Chenopodium quinoa* Willd. was also tested in order to study the potential for biogas production. In a first approach *C. quinoa* was grown with different concentrations of NaCl and the crop residues were used as substrate for biogas production. In a second approach, *C. quinoa* was also grown with different salt concentrations but fresh biomass was used as substrate. The more NaCl is in the culture medium, the higher the sodium, potassium, crude ash and hemicellulose content in the plant tissue whereas the calcium, sulfur, nitrogen and carbon content in the biomass decrease. According to this study, it is also possible to produce high yields of methane using biomass of *C. quinoa*. In another experiment, *Tripolium pannonicum* was cultivated under hydroponic conditions with different concentrations of the antimicrobial SDI with the purpose of analysing the uptake and biodegradation of SDI from the culture medium and up to the anaerobic digestion. SDI was analyzed by liquid chromatography coupled to positive ion electrospray mass spectrometry (ESI LC-MS). Based on the findings, *T. pannonicum* is able to uptake SDI. The more SDI is in the culture medium, the higher the SDI content in the plant tissue. According to this study, it is possible to produce high yields of biogas using biomass of *T. pannonicum* containing SDI and at the same time biodegradation of SDI is carried out. The highest specific biogas yield is obtained using shoots as substrate of the plants cultivated at 5 $mg \cdot L^{-1}$ SDI.

Keywords: anaerobic digestion; biogas production; *Chenopodium quinoa*; halophytes; phytoremediation; renewable energy; sulfadimidine degradation; *Tripolium pannonicum.*

Zusammenfassung

Diese Arbeit analysiert die mögliche Verwendung von Halophyten als Biofilter und ihre weitere Verwendung für die Biogasproduktion durch anaerobe Fermentation. Verschiedene Halophyten wurden getestet, um die Fähigkeit der Pflanzen zu untersuchen, mit unterschiedlichen Salzkonzentrationen unter hydroponischen Bedingungen zu wachsen. Pflanzenwachstumsparameter sowie die Abnahme von Nitraten, Phosphaten und dem Antibiotikum Sulfadimidin (SDI) wurden untersucht und anschließend wurde die Biomasse als Substrat für die Biogasproduktion eingesetzt. Die Strand-Aster (*Tripolium pannonicum*) wurde mit unterschiedlichen Salzkonzentrationen kultiviert und frische Biomasse wurde verwendet, um das Biogasproduktions-Potential zu bestimmen. Nach den Erkenntnissen ist es möglich, hohe Ausbeuten von Methan aus Halophyten zu produzieren, die in der Gegenwart von Salz kultiviert wurden. Methanerträge werden durch das Salz in dem Kulturmedium beeinflusst, mit einer optimalen Konzentration zwischen 10 $g \cdot L^{-1}$ und 30 $g \cdot L^{-1}$ NaCl. Hohe Konzentrationen von Salz in den anaeroben Reaktoren hemmen die Biogas- und Methan-Produktion. Allerdings kann durch die Verwendung von einem an Salz angepassten Inokulum die Biogasausbeute im Vergleich zu dem nicht angepassten Inokulum erhöht werden. Die salztolerante Art *Chenopodium quinoa* Willd. wurde ebenfalls untersucht, um das Potenzial für die Biogasproduktion zu testen. In einem ersten Ansatz wurde *C. quinoa* mit unterschiedlichen Konzentrationen von NaCl kultiviert, und die Pflanzenrückstände wurden als Substrat für die Biogasproduktion verwendet. In einem zweiten Ansatz wurden die Pflanzen auch mit unterschiedlichen Konzentrationen von NaCl kultiviert, aber die frische Biomasse wurde als Substrat verwendet. Je mehr NaCl in dem Kulturmedium vorhanden war, desto höher ist der Natrium-, Kalium-Rohasche und Hemicellulosegehalt im Pflanzengewebe während sich der Kalzium-, Schwefel-, Stickstoff- und Kohlenstoffgehalt in der Biomasse verringert. Nach dieser Studie ist es möglich, hohe Ausbeuten an Methan mit der Nutzung von Biomasse von *C. quinoa* zu erzeugen. In einem weiteren Experiment wurde *Tripolium pannonicum* mit verschiedenen Konzentrationen des Antibiotikums SDI unter hydroponischen Bedingungen kultiviert, mit dem Ziel die Aufnahme und den biologischen Abbau des SDI aus dem Kulturmedium bis zu der Vergärung zu analysieren. SDI wurde durch Flüssigchromatographie gekoppelt mit positiver Ionen Elektrospray-Massenspektrometrie (ESI LC-MS) analysiert. Basierend auf den Ergebnissen hat sich gezeigt, dass *T. pannonicum* in der Lage ist SDI aufzunehmen. Je mehr SDI in dem Kulturmedium ist, desto höher ist der Gehalt an SDI in dem Pflanzengewebe. Laut dieser Studie ist es möglich, hohe Ausbeuten von Biogas zur gleichen Zeit mit dem Abbau von SDI zu erzielen. Die höchste spezifische Biogasausbeute wird mit Sprossen der bei 5 $mg \cdot L^{-1}$ SDI gehaltenen Kulturpflanzen als Substrat erhalten.

Schlüsselwörter: anaerobe Gärung; Biogasproduktion; *Chenopodium quinoa*; Halophyten; Phytoremediation; erneuerbare Energie; Sulfonamid-Abbau; *Tripolium pannonicum*

Content

List of abbreviations

Acronyms

ADF om	Acid detergent fibre
ADL	Acid detergent lignin
ATP	Adenosine triphosphate
C_2H_5OH	Ethanol
$C_3H_6O_3$	Lactic acid
CA	Crude ash
$CaSO_4$	Calcium sulfate
CF	Crude fibre
CH	Total sugars
CH_3CH_2 CH_2COOH	Butyric acid
CH_3CH_2COOH	Propionic acid
CH_3COOH	Acetic acid
CH_3OH	Methanol
CH_4	Methane
CL	Crude lipids
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
FW	Fresh weight
H_2S	Hydrogen sulfide
HCEL	Hemicellulose
HCOOH	Formic acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
KCl	Potassium chloride
Kow	Octanol/water partition coefficient
LC-MS	Liquid chromatography mass spectrometry
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
Mtoe	Million tons of oil equivalent
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride

NADH	Nicotinamide adenine dinucleotide
NDF om	Neutral detergent fibre
NDL	Neutral detergent lignin
NH_4	Ammonium
NO_2	Nitrites
NO_3	Nitrates
PO_4	Phosphates
PS	Practical salinity (PSS-78)
SBY	Specific biogas yield
SDI	Sulfadimidine
SMY	Specific methane yield
TiO_2	Titanium dioxide
VS	Volatile solids
WWTPs	Wastewater treatment plants

Chapter 1

General introduction

The increase in global population has caused an increase in the demand for food, energy, soil, water, among other resources and consequently, has led to an overexploitation of natural resources. One of the main problems is the shortage and pollution of water, accompanied by an increase in salinization due to the lack of precipitation and improper water resource management practices by the users. Yet, the values of these resources are poorly understood and their management has been neglected. Thus there is a need for research in a number of areas, though this must be set in the appropriate context for the countries in which the problems occur. This leads to investigation and promotion of environmentally sound practices and techniques, as well as, research and use of other potential resources such as halophyte biomass for renewable energy production. For this reason, halophyte plants were used in order to study the biofiltering capacity and their further use for biogas production as an energy source through an anaerobic digestion process.

Soil and water resources

The rapid population growth has an 80% probability that world population will increase to more than 9.7 billion in 2050 (UN, 2015). Recent droughts and the high contamination rate reduces access to drinking water resources. Droughts, mainly due to the shortage of rain which is often erratic and poorly-distributed, heavier rainfalls leading to lower storage capacity and improper water resource management practices will increase the water scarcity, affecting surface and groundwater. It is predicted that by 2050, 67% of the world's population will live in areas where water is scarce (Wallace, 2000). Because of water scarcity and limitation of arable land, the demand of new approaches for soil and water-resource planning and management are of great importance.

As population increases worldwide, the arable land per caput also decreases (Fig. 1), while more land is becoming degraded. It is estimated that about 30% of the world's land surface, or 4.2 billion ha is suitable to some extent for rain-fed agriculture. Of this area some 1.6 billion ha is already under cultivation (Bruinsma, FAO 2009). Moreover, global food production will need to increase by 38% in the year 2025 and by 57% in 2050 (Wild, 2003) if food supply to the growing world population is to be maintained at current levels. In addition, it is also estimated that about 15% of the total land area of the world has been degraded by soil erosion and physical and chemical degradation, including soil salinization (Wild, 2003). Based on this, new

cultivation techniques and the use of other potential crops to exploit areas that are not suitable for traditional crops are necessary.

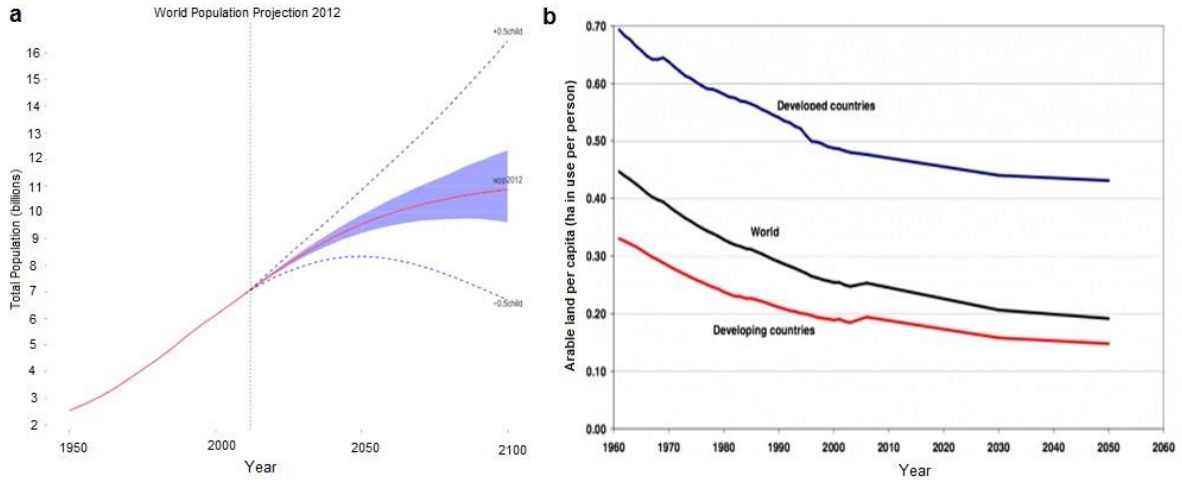

Fig. 1. World Population (a) and arable land per capita (b)

World population projection (red line) with 80% prediction interval (shaded blue area) and the traditional UN high and low variants (dashed blue lines).

Source: UN Population Division 2012 (Gerland et al., 2014) and Bruinsma (2009)

Water pollution and the improper water resources management

Water pollution is the leading worldwide cause of deaths and diseases (Mandour, 2012), which is a major global problem and requires ongoing evaluation. As Earth's population continues to grow, people are placing increasing pressure on water resources. In a sense, the oceans, rivers, and other inland waters are being polluted by human activities. Agriculture is the activity that consumes most freshwater in the world, which represents about 75% of current human water use (Wallace, 2000), and it is the main factor of degradation of surface and groundwater resources due to erosion and nutrient runoff in solution. A certain amount of chemical fertilizers used by farmers is dissolved in the run-off and transported downstream and consequently end up in rivers, groundwater and seas. Nitrogen levels in groundwater have increased in many countries as a result of the intensification and expansion of agricultural activities. Nitrate levels have grown in some countries to the point where more than 10% of the population is exposed to concentrations above the 10 $mg \cdot L^{-1}$ guideline (FAO, 1996), which can cause methaemoglobinaemia, or blue-baby syndrome in infants. Currently, aquaculture is also a problem in freshwater environments, estuaries and coasts, causing eutrophication in oceans, lakes and rivers. Eutrophication occurs when water quality deteriorates due to nutrient pollution, mainly nitrates and phosphates. Growth of aquatic weeds, algae and cyanobacteria increases greatly and this results in oxygen depletion, therefore it has caused the depletion for

other aquatic life in the ecosystem. In addition, cyanotoxins produced by cyanobacteria are powerful natural poisons which can kill many forms of life and may be a serious health hazard to humans (Carpenter et al., 1998). Domestic wastewater is another important source of contamination from a physical, chemical and microbiological viewpoint. There are up to 70,000 known and emerging chemicals that might be present in various water resources, including for drinking water production (Korostynsca et al., 2013). Moreover, many other pollutants, known to be harmful to health such as arsenic, fluoride, selenium and uranium have been found in the water. Organic compounds, which include phthalates, bisphenols, alkyl phenols, alkyl phenol ethoxylates, polyethoxylates, pesticides, human hormones and pharmaceuticals, have also been found in water (Fawell and Nieuwenhuijsen, 2003). Among this vast array of contaminants of anthropogenic origin, pharmaceutical compounds found in the water have drawn attention, since these have one of the largest inputs into the environment. For example, in 1997 in the EU the total amount of antibiotics used was 12,752 t of which 7,659 t were used in human therapy and 5,093 t were applied in the veterinary sector (Thorsten et al., 2003), and considering a reported mean degradation rate up to 60%, 40% or more of those several thousand tons of antibiotics will enter the environment. In this respect, for example, in Germany a total of 412 t of antibiotics were used in 1998, of which 305 t are discharged into wastewater (Kümmerer, 2009a).

In addition to the high contamination rates, most of the wastewater is not treated in developing countries, while in developed countries the wastewater treatment plants (WWTPs) are not able to fully remove all pollutants. As a result, many lakes as well as parts of regional seas (North Sea, Baltic Sea, parts of the Mediterranean) show significant eutrophication. For example, the International Lake Environment Committee (ILEC), in cooperation with the United Nations Environment Programme (UNEP), undertook a project entitled “Survey of the State of the World Lakes”, where data from 217 important lakes worldwide were gathered. As a result, all 217 lakes showed an increase in the level of eutrophication over the past 50 years, including Lake Constance in Germany (UNEP, 2001). This problem is of great concern and leads us to use the water resources properly. The first step is to avoid nutrient loading into the water bodies as early as possible by proper management and planning practices. A controlled use of fertilizers is very important in order to prevent or reduce nutrient inputs of diffuse sources such as surface runoff and drainage, since this is a type of pollution very complex and difficult to control. A study conducted by Carpenter et al. (1998) pointed out that nonpoint N sources are responsible for more than 90% of the N inputs to over one-half of the 86 rivers studied, while nonpoint P sources contributed more than 90% of the P in one-third of these rivers. In addition,

governments also must undertake the necessary work and create new policies focused on water pollution prevention. In this sense, the EU Water Framework Directive is the most important policy to achieve this goal (Werner, 2012) which has identified the main pollutants with their emission limit values and environmental quality standards (EC, 2000). Nevertheless, new emerging contaminants keep appearing in the aquatic environment. These new contaminants, and the fact that the chemical status of 40% of surface waters is unknown, show that current knowledge and monitoring is clearly insufficient in many member states of the European Union (Werner, 2012). In this context, wastewater also must be properly treated in order to remove most contaminants and avoid pollution of other water bodies including drinking water.

Wastewater treatment

The purpose of WWTPs has been to remove known pollutants such as nitrogen and phosphate, metals, suspended solids, pathogens and organic load but less work has been done to remove emerging pollutants such as pharmaceutical compounds. Undesirable pollutants in wastewater have varied in concentration and type of contaminant depending on the source of contamination. In developing countries of the total discharge generated by pollution sources, only a portion is collected in sewerage systems, while the rest is discharged to natural systems without any pretreatment. Wastewater is main cause of contamination of aquatic systems, therefore its treatment is important for the conservation of other systems.

Some WWTPs include three degrees of treatment: primary treatment, including grit removal, filtration, grinding, flocculation (aggregation of solids) and sedimentation; secondary treatment involves oxidation of dissolved organic matter by biological processes, which is then filtered; and tertiary treatment, which involves disinfection (using chorine, chloramines, chlorine dioxide and ozone) and advanced methods for biological nitrogen removal and also physico-chemical methods such as granular filtration, adsorption techniques by activated carbon, reverse osmosis and electrodialysis. During the treatment of wastewater there are three main types of processes: physical process which depend essentially on the physical properties of the pollutants such as particle size, specific weight and viscosity. Common examples of such processes are: screening, sedimentation, filtration, gas transfer. Some chemical processes include coagulation, precipitation, and ion exchange, while biological processes use organisms to break down organic substances and the removal of nitrogen in wastewater.

Regardless of the method used, none of the technologies can remove all the compounds. For example, phosphates and nitrates are not below acceptable limits, and it leads to major contamination of the receiving waters. In most cases, phosphorus is removed by chemical

precipitation with iron, alum or lime. Aluminum hydroxide has been used as a strong adsorption agent for orthophosphate and condensed phosphate but also calcium-phosphorus precipitation is a common method to remove phosphorus (de-Bashan and Bashan, 2004), although these techniques may produce other undesirable products. Nitrogen can be taken up by microorganisms or by plants but can also be oxidized (nitrification) or reduced (denitrification). Advanced chemical oxidation processes for the conversion of ammonia into nitrogen gas or nitrate has been reported (de-Bashan and Bashan, 2010). The treatment method depends on the contaminant to remove. Chemical precipitation is used also for metal removal, and sometimes metal recovery. After this process, the precipitates create materials that usually end up in landfills (de-Bashan and Bashan, 2010). Each treatment method has certain advantages and disadvantages. For example, some harmful by-products such as trihalonmethanes are generated during chlorination, for this reason some countries, including Germany, avoid its use. The type and quantities of these by-products depend on a number of factors such as bromine levels, content of organic matter, temperature and pH (Fawell and Nieuwenhuijsen, 2003).

It is known that conventional WWTPs are not designed to efficiency remove micropollutants such as pharmaceutical compounds and this results in their dispersion in the environment. For example, a removal efficiency up to 30% for atenolol, carbamazepine, metoprolol, trimethoprim, mefenamic acid and clofibric acid has been reported (Miège et al., 2009). Lishman et al. (2006) reported reductions of the antimicrobial Triclosan in twelve treatment plants ranging from 74% to 98%. This wide variation in the removal of pharmaceutical compounds depends on many factors. The main mechanisms involved in removal efficiency of these compounds are biodegradation, oxidation, filtration and sorption on sludge or particulate matter (Miège et al., 2009). Of these mechanisms, sorption is an important pathway for elimination of antimicrobials, which depends on the neutral or charged particles present in the media. Therefore, sorption of these organic compounds depends not only on the hydrophilicity, but also on the redox potential, pH, stereo chemical structure and chemical nature of both the sorbent and the molecule. For example, antimicrobials such as quinolones or tetracyclines are eliminated by more than 50% due to sorption to sewage sludge (Kümmerer, 2009b), while triclosan is 79% biologically degraded, 15% adsorbed to sewage sludge and 6% discharged to the receiving water (Lishman et al., 2006). The removal efficiency also depends on the physicochemical properties of the contaminant, hydraulic retention time, sludge retention time, temperature, light intensity, concentration, chemical interactions as well as the treatment method. Chemical methods with antibiotic removal rates between 55 and 70% have been reported (Fatta-Kassinos et al., 2011). An example of this treatment method is the use of

photocatalyst titanium dioxide (TiO_2) in the advanced oxidation process. The disadvantage of these advanced oxidation processes is the high operating and maintenance costs. In addition to the high costs of some chemical treatments, some other undesirable by-products may be generated, as previously mentioned. Less information is available on biological processes (de-Bashan and Bashan, 2010). Constructed wetlands technology is also becoming more important to treat wastewater. Wetlands have proven to be one of the most cost-effective method for treating wastewater (Buhmann and Papenbrock, 2013; Turcios and Papenbrock, 2014). Unlike chemical treatments or other conventional treatment methods, the operation and maintenance costs of wetlands are much lower (Vymazal, 2002). Constructed wetlands are characterized by a simple structural design, easy operation, a minimal use of external energy and high degree of water purification. The plants in a constructed wetland ensure a high evapotranspiration and thus contribute to improving the microclimate (DWA, 2014) and in addition the harvested biomass may be used for biogas production. The main limiting factor for the use of such processes is the relatively large space required (DWA, 2014). However, no single technology can completely remove all pollutants, therefore, integration of removal processes would be more effective for treating wastewater. In addition, preventing pollution is among the most cost-effective method. In order to prevent pollution of the aquatic environment, authorities should also undertake prevention and mitigation measures.

Soil Salinity

Salt-affected soils occur in all continents and under almost all climatic conditions. Worldwide, the major factor in the development of saline soils is the lack of precipitation. Most salt-affected soils are found in the arid and semiarid regions compared to the humid regions. Normally, the salinization of agricultural land affects a considerable area of irrigation projects, on the order of 20 to 30% (Fig. 2). Salinity in drylands can occur when the groundwater is close to the soil surface or due to the seawater intrusion. Seawater intrusion happens when water is drawn from aquifers more than is recharged by surface water from rainfall. The salts from the groundwater are raised by capillary action to the surface. This occurs when groundwater is saline and is favoured by improper practices.

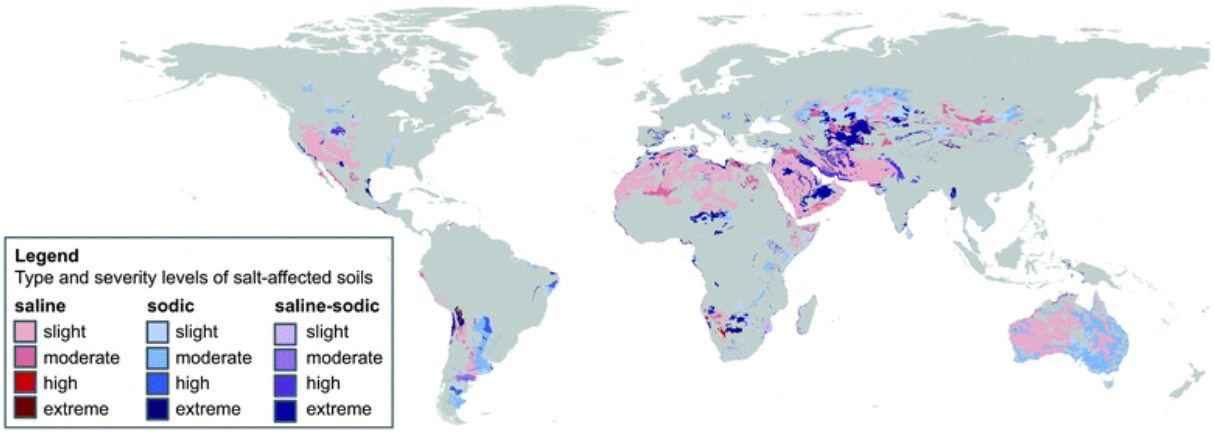

Fig. 2. Global distribution of salt-affected soils

Source: FAO (2008)

According to a more recent report published by FAO in year 2000, the total global area of salt-affected soil including saline and sodic soils was 831 million ha (Martinez-Beltran and Manzur, 2005), extending over all the continents including Africa, Asia, Australasia, and the Americas. Using the FAO/UNESCO soil map of the world (1970-1980), FAO estimated that globally the total area of saline soils was 397 million ha and 434 million ha of sodic soils. Of the then 230 million ha of irrigated land, 45 million ha (19.5 percent) were salt-affected soils; and of the almost 1 500 million ha of dryland agriculture, 32 million (2.1 percent) were salt-affected soils (FAO, 2000).

Renewable energy and biogas production

In 2010, EU Member States submitted their action plans to meet the 2020 Renewable Energy Sources targets. Wind and biomass were the most prominent renewable energy sources (Foreest, 2012). Due to this initiative, renewable energy has been steadily increasing in Europe (Fig. 3). In 2012, the European renewable energy production (177 Mtoe) overtook the production of energy from indigenous coal (167 Mtoe), natural gas (133 Mtoe) and oil (77 Mtoe) (AEBIOM, 2015).

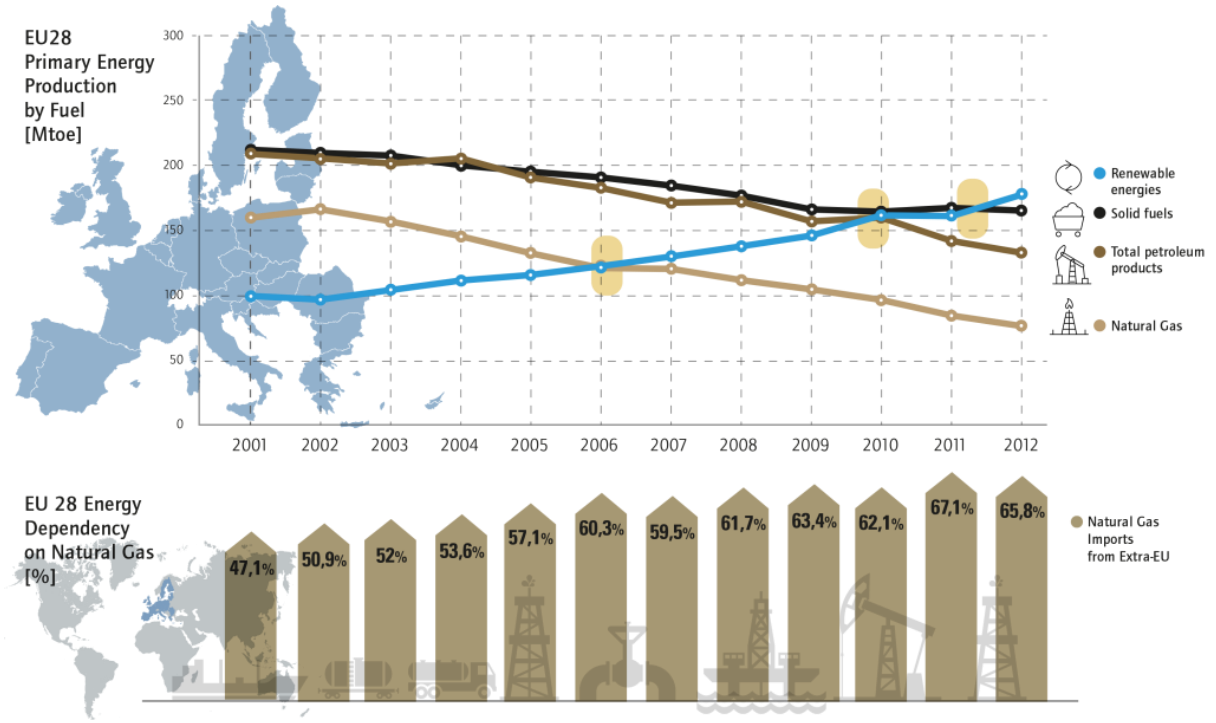

Fig. 3. Sources of primary energy production in Europe

Source: AEBIOM, 2015

Biomass plays a fundamental role in the production of renewable energy. The term biomass refers to organic material which can be used for the production of biofuels or biogas. The biomass for biogas production through anaerobic digestion is obtained mainly from agricultural and livestock waste, and from energy crops such as maize, which is most widely used in biogas plants in Germany (Fig. 4). The anaerobic digestion process is one of the most suitable for energy production and at the same time the digestate may be used as fertilizer.

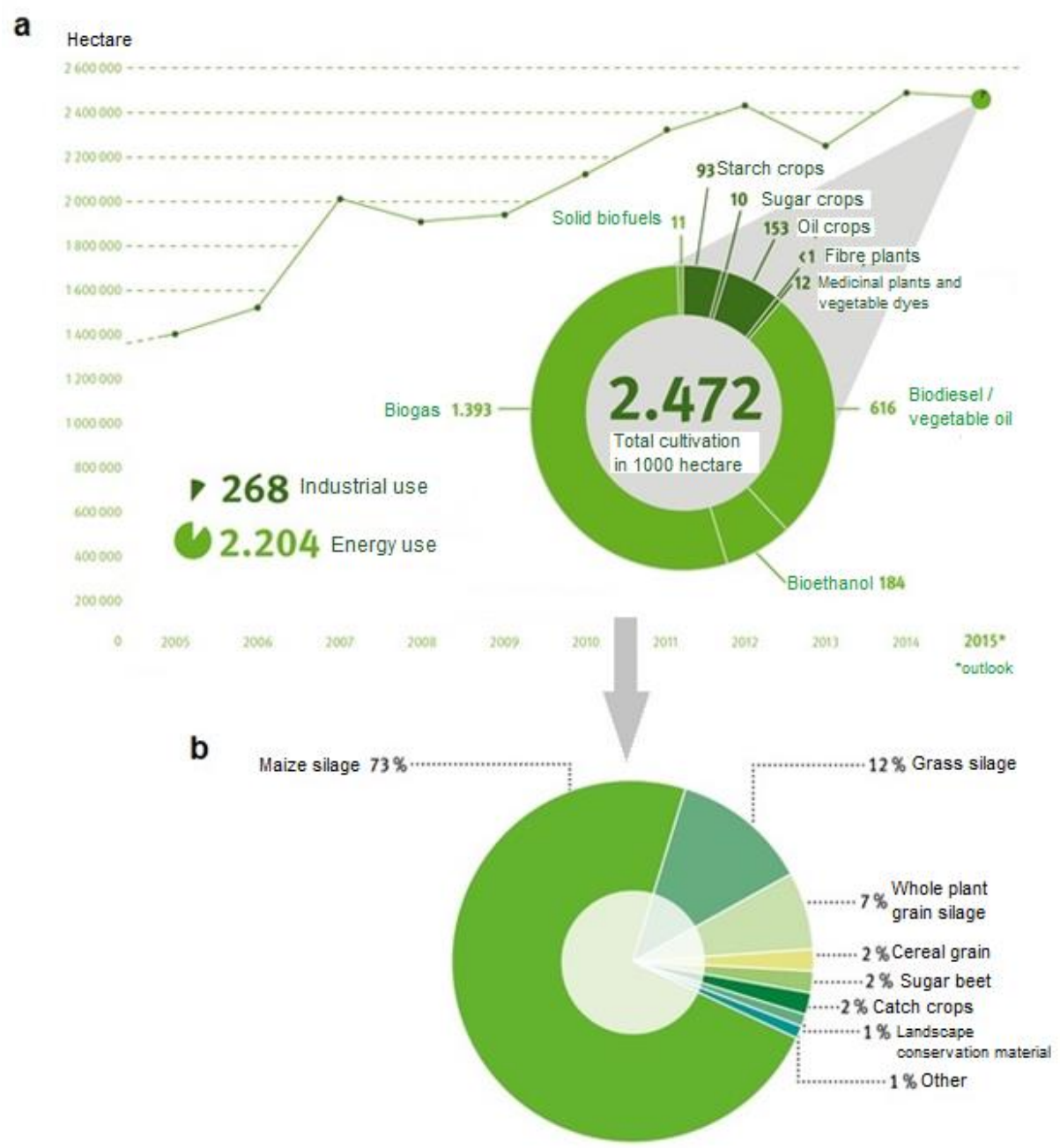

Fig. 4. Cultivation of renewable resources (a) and substrates used in biogas plants in Germany (b)
Source: FNR, 2015

The anaerobic digestion process is carried out by different microorganisms (bacteria and archaea) in the absence of oxygen, which decompose the organic matter into biogas (CH_4, CO_2, H_2, H_2S) and digestate (material remaining after the anaerobic digestion). The decomposition of organic matter occurs in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 5).

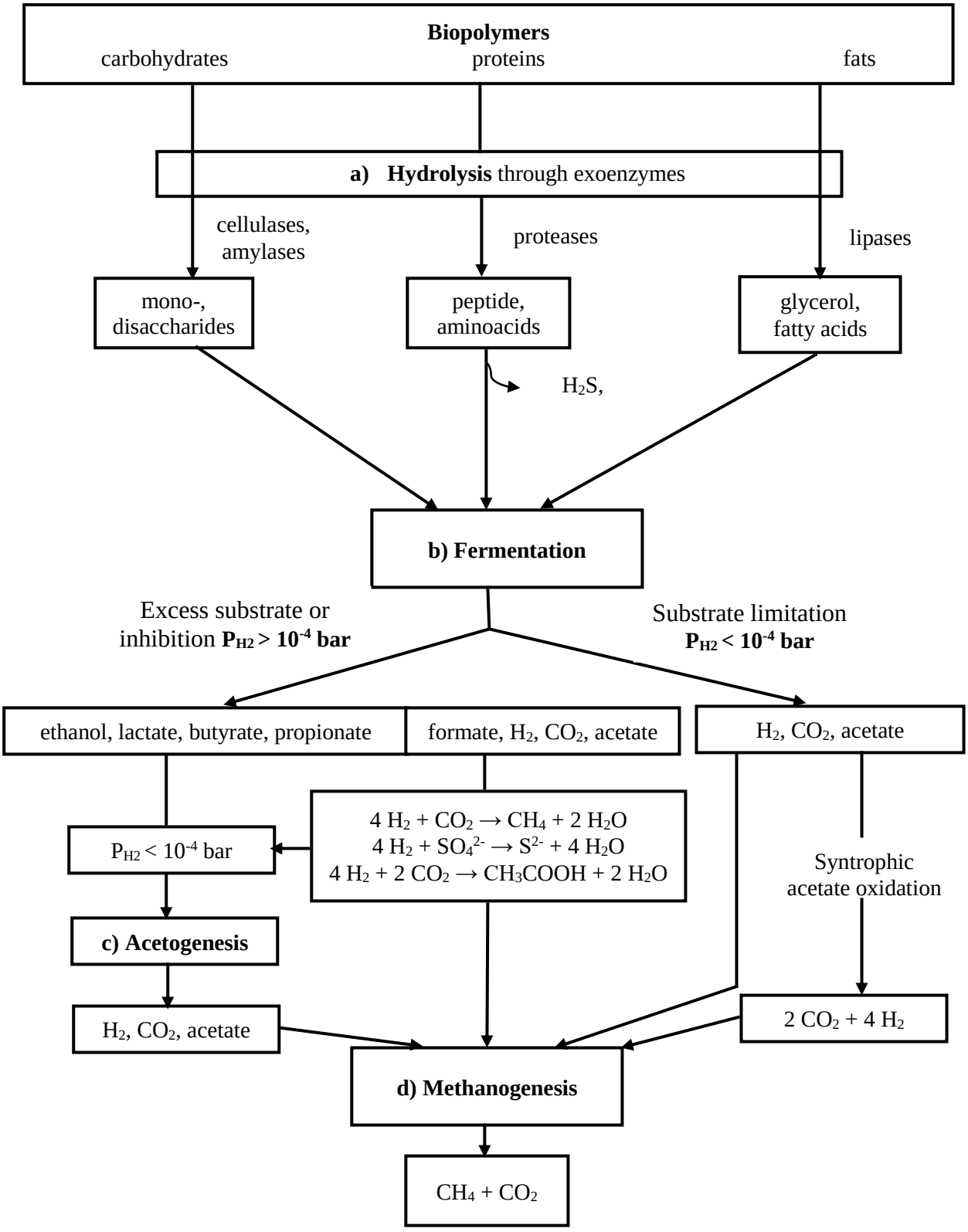

Fig. 5. Schematic representation of the anaerobic food chain starting from a biopolymer which is degraded through (a) hydrolysis, (b) fermentation, (c) acetogenesis and (d) methanogenesis to the end products CO_2 and CH_4 (biogas).

Source: Gallert et al. (2015)

During the first stage, hydrolysis, bacteria transform the organic substrate into soluble monomers and polymers. Proteins, carbohydrates and fats are transformed into amino acids, monosaccharides and fatty acids, respectively. Hydrolysis is catalyzed by enzymes excreted from the bacteria, such as cellulase, protease, and lipase. In the second stage, acidogenesis, acidogenic bacteria turn the products of hydrolysis into short chain volatile fatty acids, ketones, alcohols, hydrogen and carbon dioxide. The principal acidogenesis stage products are propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), acetic acid (CH_3COOH), formic acid ($HCOOH$), lactic acid ($C_3H_6O_3$), ethanol (C_2H_5OH) and methanol (CH_3OH), among others. In the methanogenesis stage, anaerobic archaea converts hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide (de Mes et al., 2003; Demirel and Scherer, 2008; Gallert et al., 2015).

The current biogas production and long-term outlook varies across Europe. Factors such as renewable energy policy, regulatory framework, land availability, prices, the availability of natural gas and biomass determine the success of this important renewable energy source. During the last decade, the amount of biogas produced in Europe has been increasing despite the recent years it has slowed down in Germany (Fig. 6).

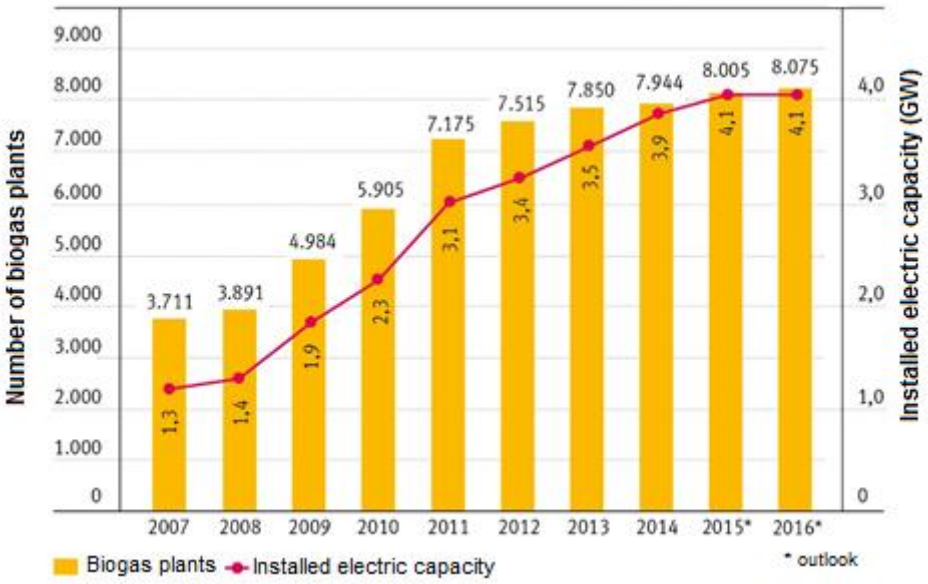

Fig. 6. History of biogas plants in Germany

Source: FNR, 2015

Foreest (2012) reported that in 2010, primary production of biogas in Europe was 10.9 Mtoe, representing an increase of 31% compared to 2009. The projected amount of biogas varies depending on many factors as mentioned above. The Institute for Energy and Environment in

Leipzig calculated a theoretical potential for Europe of 166 Mtoe in 2020, while AEBIOM estimated the biogas potential in terms of biomass origin with an estimated potential of almost 40 Mtoe in 2020. Germany, as current leader in biogas development in Europe, in 2020 will produce a projected biogas amount of 5.3 Mtoe in terms of gross energy production (Foreest, 2012), and a bioenergy potential of 26% by 2050 (Fig. 7).

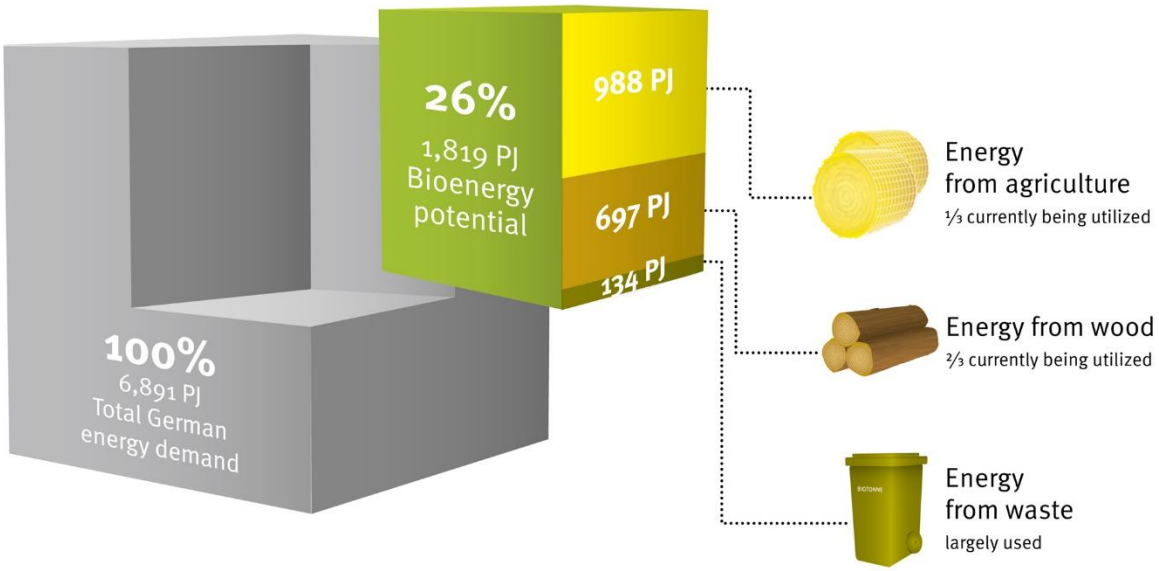

Fig. 7. Bioenergy potential in Germany in year 2050
Source: FNR, 2016

Competition between food and biofuel production

The controversy between the use of cultivated plants for biofuel production instead for food production is an issue that has been debated for a long time. This is mainly due to the interaction of several factors that can positively or negatively affect the price system. Moreover, land and water resources are limited, thereby creating frictions and competition for these limited resources, and raising concerns about food security.

Biofuel production, except when based on crop residues and waste, requires land, therefore it competes with other economic and agriculture activities, urbanization and with some environmental objectives, especially protection of biodiversity and carbon sequestration (HLPE, 2013). According to Foreest (2012), in Germany between 90-95% of all biogas plants use crops such as maize, grass and cereals, mainly in combination with manure. Based on this, biomass from energy crops is one important factor for biogas production. As mentioned above,

land and water are limited resources, consequently the biomass obtained from energy crops depends in part on land and water availability. Although Foreest (2012) reported that the amount of land devoted for growing crops for energy purposes is only 0.19% of the world's total land area and only 0.5-1.7% of global agricultural land. However, generally speaking, as the production of energy crops increases, decreases the amount of available land for food crops and also the supplied quantity of grains, as is the case of maize, therefore the quantity demanded for basic grains rises, combined with an increase in prices (Fig. 8).

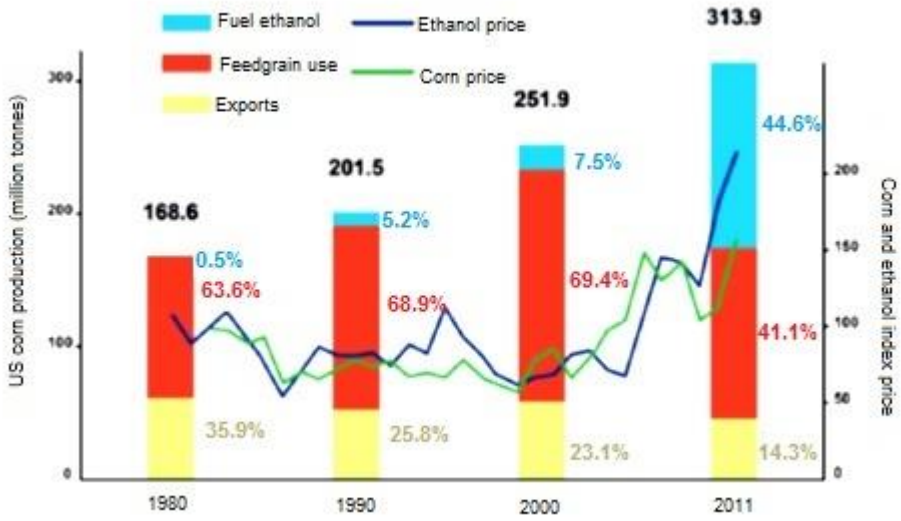

Fig. 8. Corn and ethanol prices, and US corn production
Source: HLPE, 2013

Halophyte species and their salt tolerance mechanisms

Halophytes are plants that tolerate high concentrations of soluble salt in their environments. Unlike glycophytes, halophytes are able complete their life cycle in at least 200 mM salt (Flowers et al., 2010). Halophytes represent about 2% of world angiosperm species. Of the total halophytic species, 57% came from just 13 families. The Amaranthaceae family (now including the former goosefoot family Chenopodiaceae) has the largest number of halophyte species, over half of its 550 species are salt-tolerant. The families Poaceae, Fabaceae and Asteraceae also have a large number of halophytes, although they represent fewer than 5% of the species in these families (Glenn et al., 1999). Salt tolerant species such as *Spartina alterniflora, Plantago* spp., *Triglochin* spp., *Sporobolus viginicus, Salicornia* spp., *Salsola kali, Atriplex canescens, Suaeda maritime, Batis maritima, Tripolium pannonicum, Chenopodium quinoa*, among many others have been reported (Buhmann and Papenbrock, 2013; Glenn et al., 1999).

As previously described, halophytes are able to grow in saline environments which are normally dominated by NaCl, but may contain other salts such as Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCI and Na_2CO_3. This remarkable tolerance is complex since it is determined by a number of factors, specific for each particular species (Shabala and Mackay, 2011). Salt tolerance involves physiological and biochemical adaptations for maintaining protoplasmic viability while cells compartmentalize electrolytes. Salt avoidance involves structural and physiological adaptations to minimize salt concentrations in the cells or physiological exclusion by root membranes (Koyro et al., 2008). Water potential plays an important role in the halophyte cells, which has to be lower within than outside the plasmalemma to retain cellular water and the necessary osmotic adjustment is achieved mainly by Na and C1 ions (Flowers, 1985). Na^+ and Cl^- are largely compartmentalised in vacuoles in halophytic plants (Flowers et al., 2010), which contains 90% or more of cell water. Na^+ must be actively pumped into the vacuole from the cytoplasm and it appears to be mediated by Na^+/H^+-antiporters in the tonoplast, working together with H^+-ATPases and perhaps PP_iases that provide the proton motive force (Glenn et al., 1999). Nevertheless, excessive ion content in the cytoplasm should be avoided and this is apparently achieved by mechanisms that regulate K^+/Na^+ selectivity. Some genes may also be involved in salt tolerance in halophytes such as the plasma membrane H^+-ATPase gene (Niu et al., 1993). The water potential in the cytoplasm of halophytic plants is also adjusted by the accumulation of organic solutes (Glenn et al., 1999). Some halophytes accumulate proline as a nontoxic and protective osmolyte under saline conditions (Parida and Das, 2005) as well as ascorbate, phenols, flavonoids and total antioxidant capacity can be increased under saline conditions (Boestfleisch et al., 2014). Taking advantage of the ability of halophyte plants to grow in saline soils, where other conventional crops such as maize cannot grow, salty environments can be exploited to cultivate these salt-tolerant plants and the biomass can be used for energy production. In addition, taking into consideration the freshwater scarcity, these plants can be watered with salty water or seawater.

Importance of halophyte plants

The population is increasing worldwide and in turn, the arable land per caput decreases (Fig. 1), while more land is becoming degraded, with competition between food crops and energy crops. As previously mentioned, it is estimated that about 15% of the total land area of the world has been degraded by soil erosion and physical and chemical degradation, including soil salinization (Wild, 2003). Currently, salt-affected soils are naturally present in more than 100 countries of the world where many regions are also affected by irrigation-induced salinization

(Rengasamy, 2006). Saline soil or salty water reduces the ability of the plant to uptake water and, consequently, inhibits plant growth. Therefore, new cultivation techniques and the use of other potential crops to exploit areas that are not suitable for traditional crops are necessary. Based on this, halophytes play an important role since these are salt-tolerant plants and can be cultivated in saline soils, in coastal areas, for treating saline wastewater and indeed can be watered with seawater. Halophytes also have high potential as crops like *Salicornia* spp. and *Chenopodium quinoa*. The former can be used as forage, biogas production or as salty vegetable which is one of the most popularly used in culinary seafood dishes, while *C. quinoa* has caught the attention due to its edible seeds with an outstanding protein quality and a high content of vitamins and minerals. To use halophytes as crops some basic conditions must be met such as the biomass yield. In this regard, some studies have documented high biomass yield of halophyte species such as *Spartina alterniflora*, which produces up to 40 $t \cdot ha^{-1}$ of biomass, *Salicornia bigelovii* produces from 12.7 $t \cdot ha^{-1}$ to 24.6 $t \cdot ha^{-1}$ of biomass and 1.39 $t \cdot ha^{-1}$ to 2.46 $t \cdot ha^{-1}$ of seed over a 200-day growing cycle, *Atriplex* spp. produces from 12.6 $t \cdot ha^{-1}$ to 20.9 $t \cdot ha^{-1}$ of biomass containing 9.9% to 19.5% protein on full-strength seawater (Glenn et al., 1999), while in *C. quinoa* seed and biomass yields up to 4 $t \cdot ha^{-1}$ and 15 $t \cdot ha^{-1}$ have been reported respectively (Jacobsen, 2003). These studies show that halophytes can produce high biomass in comparison to other conventional crops even at high salinity.

Description of the halophytes *Chenopodium quinoa*, *Tripolium pannonicum* and *Salicornia* spp.

Chenopodium quinoa Willd. (family Amaranthaceae) is a facultative salt-tolerant plant which is cultivated mainly for its edible seeds. The nutrient composition is favourable compared with common cereals. It has been selected by FAO as one of the crops destined to offer food security in the next century (Jacobsen, 2003). This species has been used for different purposes for example, industrial use of starch, protein and saponin, green fodder, animal feed, pasta, breakfast cereals, and cookies for people allergic to gluten and for diabetic patients due to the low glycemic index (Jacobsen, 2003; Vega-Gálvez et al., 2010). The grain contains about 48% starch, an average of 18% protein, which is high in lysine and other essential amino acids, 4-9% of unsaturated fat, and good quantities of calcium, phosphorus, and iron (Vaughn and Geissler, 2009). After harvest, the crop residues have high potential for biogas production (Turcios et al., 2016b).

The species *Tripolium pannonicum*, belongs to the Asteraceae family and it is a perennial growing up to 50 cm. It is also a salt-tolerant species, which grows very well at sea water concentrations, consequently it is normally found in salt marshes and estuaries. This plant

species has a high potential as biofilter to uptake compounds from water such as nitrates, phosphates (Buhmann and Papenbrock, 2013), and organic compounds like sulfadimidine (Chapter 5). Its biomass also has a high biogas potential in comparison to other energy crops (Turcios et al., 2016a).

Salicornia is a genus of an annual, leafless, succulent halophyte in the family Amaranthaceae which grows in salt marshes, on beaches, and among mangroves. The species of *Salicornia* are widely dispersed in Eurasia, North America and South Africa, ranging from the subtropics to subarctic regions. Some species like *S. bigelovii* Torr. has been evaluated as a new forage and oilseed crop for saltwater and seawater-irrigated agriculture in the coastal deserts of Mexico (Bashan et al., 2000). *Salicornia* spp. is also suitable for cultivation as a vegetable in highly saline environments and as a source of valuable secondary compounds. This species can be grown in constructed wetland systems and irrigated with nutrient-rich saline water or effluents from aquaculture (i.e. as a biofilter) to increase sustainability (Singh et al., 2014), and the biomass also has high potential for bioenergy production.

Fig. 9. Halophyte plants, (a) *Chenopodium quinoa*, (b) *Tripolium pannonicum* and (c) *Salicornia* spp.

Use of halophytes as biofilter

Agriculture and livestock activities have also intensified in recent years, as described above. Changes in these systems have generated undeniable socio-economic achievements but have created major environmental problems. Therefore, wastewater treatment using well-established and a cost-effective methods is of great importance. Biofiltration is an efficient method for treating wastewater and is also used in aquaculture as a way to minimize water replacement while increasing water quality (Turcios and Papenbrock, 2014). Plants used for biofiltering, either in natural or constructed wetlands, are gaining importance for treating wastewaters from industry, agricultural, aquaculture and runoff water (Buhmann and Papenbrock, 2013). This system has low maintenance and operating costs and, in addition, contributes to the improvement of surrounding microclimate, providing a suitable habitat for other organisms (DWA, 2014). In this context, since wastewater normally contains dissolved salts, halophyte plants are proposed for biofiltering.

As described previously, pharmaceutical products dissolved in wastewater such as antimicrobials are gaining importance, since normally the WWTPs are not able to remove these compounds from water. Their presence in the environment may result in increased drug resistance of bacteria. Resistance to antibiotics is a major public-health problem and antibiotic use is being increasingly recognised as the main problem (Kim and Aga, 2007). Antibiotics that generate greater problem in the environment belong to groups of tetracyclines, penicillins, cephalosporins, macrolides, quinolones and sulfonamides, presenting these groups major problems of bacterial resistance (Christian et al., 2003). As an alternative to decrease the antibiotics in water or soil, halophyte plants can be used as biofilter to decrease these organic compounds (Chapter 5). This system has low investment and maintenance costs and adapt well to changes in flow and pollutant load discharges, making it well suited for rural areas, livestock or agricultural industries. Moreover, the biomass obtained from the plants acting as biofilter may be used as substrate for biogas production, making it a sustainable and effective system (Fig. 10).

Use of halophytes for biogas production

The increased demand and scarcity of food, which inevitably means an increase in food prices, arises partially due to new demands on agriculture for biomass as feedstock in biogas production. Scarcity of these resources is exacerbated as demand increases mainly due to industrial uses, an increase in population and use in the production of biofuels (Bruinsma, FAO 2009). Besides this, there are other causes that can decrease the availability of these resources

such as climate change. This brings us to exploit renewable energy using sustainable systems and thus the preservation of resources for future generations is guaranteed.

In order to use a sustainable system, avoiding the competition between food crops and energy crops, biomass from halophyte plants has a high potential for biogas production. In this context, marginal land and saline water resources could be used to cultivate plants for biogas production. There are a number of annual and perennial species among halophytes that are able to grow in highly saline environments such as Sea Aster (*Tripolium pannonicum*), *Salicornia* spp., *Chenopodium quinoa*, among many other species, as described above. These halophytes produce similar amounts of biogas in comparison to other energy crops like maize (Chapter 3 and 4)

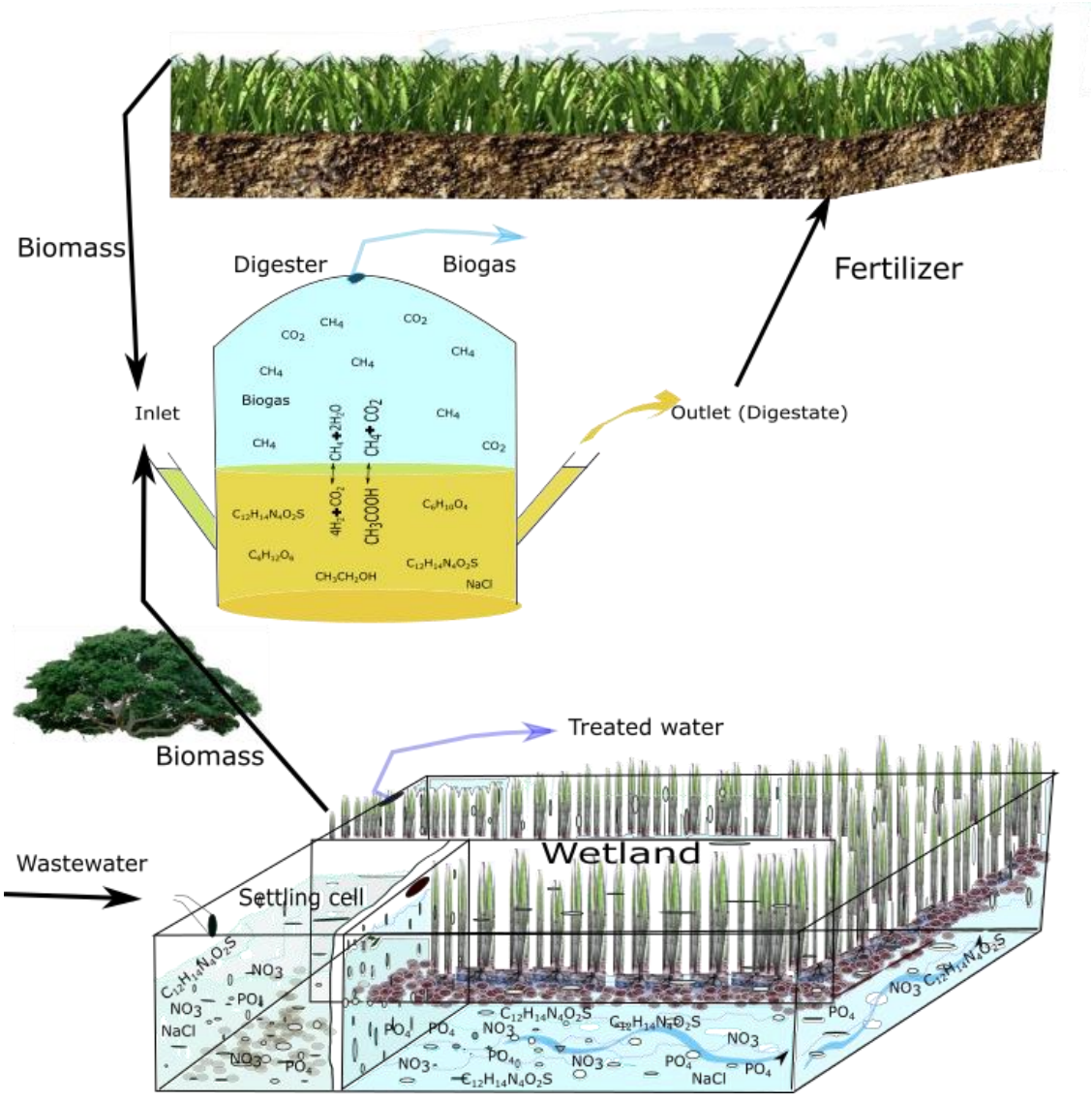

Fig. 10. Biofiltration and biogas production system

Aims of this thesis

The overall goal of this research is to study the ability of halophytes acting as biofilter to uptake organic and inorganic contaminants from the water and their subsequent use for biogas production through the anaerobic digestion process.

Specific objectives:

To study the ability of halophytes to grow exposed to different salt concentrations under hydroponic conditions. Halophytes are salt tolerant plants which can be used as a biofilter to uptake some pollutants from wastewater.

To find the reusability of halophytes after their application as a biofilter, mainly as substrate for biogas production through anaerobic digestion.

To study the salt inhibition of the biogas and methane yields using biomass from halophytes containing different amounts of salt. Halophytes are able to accumulate salt in its tissues, and salt may reduce or inhibit the biogas production.

To obtain a salt adapted inoculum in order to avoid a decrease of biogas production due to the high concentration of salt in the biomass.

To quantify the efficiency of the plant species *Tripolium pannonicum* to uptake the antimicrobial sulfadimidine. Antibiotics are a concern in water and their bioaccumulation in food products is a public health and regulatory issue, therefore biofiltration is of great importance

To determine the biodegradation of the antimicrobial sulfadimidine through the halophyte *T. pannonicum* and the anaerobic digestion process. Degradation of antibiotics using an environmentally friendly and feasible method is relevant in order to avoid bacterial drug resistance

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Chapter 2

Turcios AE & Papenbrock J, (2014): Sustainable treatment of aquaculture effluents – what can we learn from the past for the future?, Sustainability, 6, 836-856. doi:10.3390/su6020836

Sustainability **2014**, *6*, 836-856; doi:10.3390/su6020836

OPEN ACCESS

sustainability

ISSN 2071-1050

www.mdpi.com/journal/sustainability

Review

Sustainable Treatment of Aquaculture Effluents—What Can We Learn from the Past for the Future?

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Received: 23 December 2013; in revised form: 24 January 2014 / Accepted: 8 February 2014 / Published: 20 February 2014

Abstract: Many aquaculture systems generate high amounts of wastewater containing compounds such as suspended solids, total nitrogen and total phosphorus. Today, aquaculture is imperative because fish demand is increasing. However, the load of waste is directly proportional to the fish production. Therefore, it is necessary to develop more intensive fish culture with efficient systems for wastewater treatment. A number of physical, chemical and biological methods used in conventional wastewater treatment have been applied in aquaculture systems. Constructed wetlands technology is becoming more and more important in recirculating aquaculture systems (RAS) because wetlands have proven to be well-established and a cost-effective method for treating wastewater. This review gives an overview about possibilities to avoid the pollution of water resources; it focuses initially on the use of systems combining aquaculture and plants with a historical review of aquaculture and the treatment of its effluents. It discusses the present state, taking into account the load of pollutants in wastewater such as nitrates and phosphates, and finishes with recommendations to prevent or at least reduce the pollution of water resources in the future.

Keywords: aquaculture; aquaponics; halophytes; nutrients; *Salicornia* spp.; wastewater; wetlands

1. Introduction and Aims of the Review

Worldwide, there is a growing contamination of soil and irrigation water, caused, among other reasons, by intensive agricultural use and environmentally-unfriendly activity, which is due to the need to generate ever greater quantities of food to meet the demands of the growing population.

Today, aquaculture is growing rapidly: according to the FAO [1], aquaculture provides 47% (51 million tons) of the global human fish consumption. In order to keep up with population growth and increasing *per capita* fish consumption, aquaculture output is set to increase by a further 60%–100% over the next 20–30 years. In 2015, the production from aquaculture will be 74 million tons [1]. More than 40% of the world population lives not more than 100 km away from the coastlines, putting high pressure on the coastal ecosystems. Aquacultures as monocultures have been developed in the last decades, from keeping fish in ponds for easier harvesting to high technological fish farms extensively using feed, hormones and often antibiotics with a known impact on the environment. To achieve sustainability, it is necessary to intensify the production using technologies such as water recirculation systems and proper treatment to optimize this valuable resource. Further, it is important to reduce the pressure on the coastlines and produce large amounts of fish also in inland aquaculture systems close to consumers. In recent years long-forgotten historical approaches have been recovered and adapted to new technologies, such as the parallel production of fish with filter feeders and plants or algae, even in multi-trophic systems [2,3]. This concept is applicable to many standard aquaculture installations, such as ponds or net cages.

With respect to the pollution generated by aquaculture, nitrogen and phosphorus are considered as waste components of fish farming, causing serious environmental problems. In addition, several fish excrete nitrogenous waste products by diffusion and ion exchange through the gills, urine and feces. Decomposition and reuse of these nitrogenous compounds is especially important in aquaculture using recirculation systems due to the toxicity of ammonia and nitrite and the chance of hypertrophication of the environment by nitrate [2].

All aspects of water treatment play a significant role in intensive fish production, because the control and monitoring of water quality is of vital importance to the success or failure of the production. It is therefore necessary to develop new research applications focused on avoiding or at least reducing the negative impacts of aquaculture effluents on the environment. This review aims at giving an overview about aquaculture systems developed in historical times which could still be valuable for the future, about the present problems, and about innovative ideas, especially with respect to the integration of halophytic plants as biofilter in saline aquaculture systems.

2. Systems Combining Aquaculture and Plants

Several systems for combining aquaculture and biofiltering plants exist at different levels of more or less sophisticated techniques. The simple co-culture of different fish species from the same trophic level has been practiced for a long time and is known as aquatic polyculture. These

organisms share the same biological and chemical processes. The culture systems show only a few synergistic benefits. Some traditional polyculture systems incorporate a greater diversity of species, occupying several niches as extensive cultures within the same pond [4,5].

A more advanced system is the integrated multi-trophic aquaculture (IMTA). Here, the by-products or waste from one species are recycled to become inputs as fertilizers or food for another. The term "multi-trophic" refers to the incorporation of species from different trophic or nutritional levels in the same system and this is one potential distinction from polyculture systems [6]. The "integrated" in IMTA refers to the more intensive cultivation of the different species in proximity of each other, connected by nutrient and energy transfer through water. Ideally, the biological and chemical processes in an IMTA system should be balanced. This is achieved through the appropriate selection and ratios of different species providing different ecosystem functions. The co-cultured species are typically more than just biofilters; they are harvestable crops of commercial value. A working IMTA system can result in greater total production based on mutual benefits for the co-cultured species and improved ecosystem health, even if the production of individual species is lower than in monoculture over a short term period [3,7,8].

Recirculating aquaculture systems (RAS) recycle water by running it through filters to remove fish waste and food and then recirculating it back into the tanks. This saves water and the waste gathered can be used in compost or, in some cases, could even be treated and used on land. Aquaponics is a food production system that combines conventional aquaculture practices or RAS, *i.e.*, raising aquatic animals such as snails, fish, crayfish or prawns in tanks, with hydroponics, *i.e.*, cultivating plants in water, in a symbiotic environment [9,6]. In conventional aquaculture, excretions from the animals being raised can accumulate in the water and increase toxicity. In an aquaponics system, water from an aquaculture system is fed to a hydroponic system where the by-products are broken down by bacteria into nitrate and ammonium which are utilized by the plants as nutrients. The water is then recirculated back to the aquaculture system. As existing hydroponic and aquaculture farming techniques form the basis for all aquaponics systems, the size, complexity, and types of foods grown in an aquaponics system can vary as much as any system found in either distinct farming discipline [6,9,10].

3. Historical Overview of Aquaculture and Treatment of Its Effluents

Aquaculture systems have already been invented by the indigenous inhabitants of Australia. They may have raised eels as early as 6,000 BC by developing about 100 km^2 of volcanic floodplains into a complex of channels and dams using woven traps to capture eels and preserve them to eat all year round. The Japanese cultivated seaweed by providing bamboo poles, nets and oyster shells to serve as anchoring surfaces for spores [11]. Aquaponics also has ancient roots, although there is some debate on its first occurrence. First examples of aquaponics systems are found in South China and Thailand where rice was cultivated and farmed in paddy fields in combination with fish. These polycultural farming systems existed in many Far Eastern countries where fish, such as the swamp eel, common and crucian carp, as well as pond snails were raised in the paddies [4]. The Aztecs cultivated agricultural islands in Mexico as early as

1150–1350 BC where plants were raised on stationary (and sometimes movable, "floating gardens") islands in lake shallows, and waste materials dredged from canals and surrounding cities were used to manually irrigate the plants. This method of early agriculture, called *chinampa,* usually measured roughly 30 × 2.5 m or even up to 91 × 9.1 m in Tenochtitlan. The agricultural output of the *chinampas* allowed the postclassic Aztec civilization to flourish. *Chinampas* were created by staking out the shallow lake bed and then fencing in the rectangle with wattle. The fenced-off area was then layered with mud, lake sediment, and decaying vegetation, eventually bringing it above the level of the lake. Often trees such as the willow *Salix bonplandiana* (H.B.K.)-Kunth, and the cypress *Taxodium mucronatum* Ten.*,* were planted at the corners to secure the *chinampa*. Canals navigated by canoe surrounded the islands and were used to raise fish. Waste from the fish fell to the bottom of the canals and was collected to fertilize plants. These stationary or floating gardens had very high crop yields with four (or up to seven) harvests a year [12].

The development of modern aquaponics is often attributed to the various works of the New Alchemy Institute at the North Carolina State University where researchers developed the use of deep water culture hydroponic grow beds in a large-scale aquaponics system in the 70s [6]. Actually, the inorganic compounds in aquaculture systems comply to a large extent with the nutrient requirements of plants and algae. Thus, the potential of process water from RAS for plant cultivation is obvious. Approaches are dated back to 1978 and 1984, when Lewis and co-workers [13] and Watten and Busch [14] combined the production of tilapia and tomatoes. The combination of fish and plant culture where the plants not only act as biofilter but also as food for humans for examples as vegetable, salad, nutraceutical *etc.* dictates that hormones and chemicals cannot be applied. Also, the sizes of aquaponic systems were optimized and adapted to local use. For example, Canada first saw a rise in aquaponic setups throughout the 90s, predominantly as large commercial installations raising high-value crops such as trout and lettuce. Findings were made on rapid root growth in aquaponic systems and on closing the solid-waste loop. It was found that owing to certain advantages in the system over traditional aquaculture, the system can run well at a low pH level, which is favoured by plants but not fish. The commercially sized system was adapted to a smaller-scale prototype that can be operated by families, small groups or restaurants [9]. The newest approach in marine aquaculture in the 21st century is to develop the necessary parameters for the design and construction of an integrated marine recirculation aquaculture system (IMRAS) using different halophyte species [15]. Modern technical filter technologies and long practiced hydroponic systems are combined in a very efficient, hygienic and sustainable way with almost any exchange of water. The reduction of exchanging process water makes the systems ecologically more sustainable and economically more successful.

4. Present State

Water is one of the most abundant compounds in nature and covers approximately three quarters of the surface of the earth. Over 97% of the total water on the planet is in the oceans and other saltwater bodies, and its use is restricted. Of the remaining 3%, above 2% is in the solid state, which makes it practically inaccessible. Therefore, only the remaining 0.62% found

in lakes, ponds, rivers and groundwater is available for human use such as industrial and agricultural activities. The main problem is its patchy distribution across the planet [16]. The primary renewable source of freshwater is continental rainfall, which generates a global supply of 40,000–45,000 km^3 per year. This more or less constant water supply must support the entire world population, which is steadily increasing by roughly 85 million per year [17]. Thus, the availability of freshwater *per capita* is decreasing rapidly.

The immoderate use of natural resources has a negative effect on the ecosystems from which they are obtained and ecosystems in which they are used. The case of water is one of the best examples; more water consumption by human beings leads to an increase in wastewater discharges. From the total of this contaminated water, only a portion is collected in treatment plants, while the rest is discharged to natural systems directly without any pretreatment. It is necessary to establish purification systems before discharging as an important measure for the conservation of the systems [16]. In this context, aquaculture is an activity that requires a high volume of water and therefore a considerable amount of wastewater is discharged. The accumulation of excreta and food waste during fish culture often causes a deterioration of water quality, with negative effects on the fish and on the environment. This wastewater contains considerable amounts of nitrogen, phosphorus and organic matter [18], and can degrade other water bodies. Therefore, an appropriate wastewater treatment process is vital to prevent negative impacts on the surrounding aquatic environment—such as hypertrophication—and for sustaining aquaculture development worldwide.

4.1. Wastewater Management

A number of physical, chemical and biological methods used in conventional wastewater treatment have been applied in aquaculture systems. Solids removal is accomplished by sedimentation, sand or mechanical filtration. Biological processes such as submerged biofilters, trickling filters, rotating biological contactors, and fluidized bed reactors are employed for the oxidation of organic matter, nitrification, or denitrification [19]. Rotating microscreens are commonly used in land-based intensive fish-farms in Europe, with a screen mesh pore size of 60–200 μm [18]. These methods do help with phosphorus removal but are costly in terms of capital investment, energy consumption and maintenance requirements; however, little research has been focused on aquaculture wastewater.

Researchers have demonstrated that wetland systems can remove significant amounts of suspended solids, organic matter, nitrogen, phosphorus, trace elements and microorganisms contained in wastewater [20]. The aims of waste treatment and solids management differ, depending on whether the intensive culture system is single-pass flow-through, water reuse with little exchange, or a recirculating water system, as summarized by Losordo and Westers [21]. Removal of solids, organic matter, ammonia and nitrite are critical for the development of recirculating aquaculture systems [22]. In these systems, fish can be cultured next to other organisms, which are converting otherwise discharged nutrients into valuable products [23], and therefore make the system feasible.

4.1.1. Solids Loads

In order to maintain the total suspended solids (TSS) at acceptable levels for discharging or recycling, it is important to understand the nature of the waste. Appropriate management practices and/or treatment technology can then be applied as described by Cripps and Bergheim [18]. Many studies and reviews, including Cripps and Kelly [24], have shown that aquaculture waste characteristics are not conducive to easy treatment, because of their low concentrations in the effluent. Fish-farm operations have changed in recent years, due to an intensification of farming. These changes involve an increase in culture density and a decrease in specific water consumption. There have also been improved feeding formulations and systems that reduce losses through runoff. The addition of dietary binders to fish feed, such as *Alginate* and *Guar gum*, significantly enhances the stability of fish feces thus favoring the formation of large waste particles with high mechanical removal potential and a considerably improved leaching resistance. These binders have no negative side effects on the health of the fish and digestibility of macronutrients [25]. Supporting this, data presented by Kelly *et al.* [26] and Bergheim *et al.* [27] showed that treatment efficiency, in terms of the separation of particles from the effluent, increased with increased solids concentration; the settling efficiency of an aquaculture sludge sedimentation chamber increased from about 58% at about 1 mg suspended solids (SS) min^{-1} to nearly 90% at 18 mg SS min^{-1} at the same flow rate.

This indicates that aquaculture waste solids are difficult to treat, and that by increasing the concentration prior to treatment, an increase in treatment efficiency, or clarification rate, can be expected [18].

Generally, solid concentrations in the untreated effluent from flow-through farms are around 5–50 mg L^{-1}, and do not appear to have altered greatly within the last 20 years. This was shown by Hennessy *et al.* [28], Bergheim *et al.* [29] and Cripps [30] who reported a wide range of total solid concentrations of 1.6–14.1, 0–20.1, and 6.9 mg L^{-1}, respectively. However, these concentrations can vary widely depending on the management of aquaculture systems.

4.1.2. Nutrient Load

The pollution load in wastewater is variable, it depends on several parameters. Kelly *et al.* [31] found that the waste quantity discharged from a fish farm is directly related to temperature. Foy and Rosell [32] showed that the proportion of nutrients in the particulate fraction increased with temperature. This relationship is based on the fact that an increase in temperature also increases the rate of metabolism. In integrated intensive aquaculture systems, the waste load such as nitrates and phosphates can be reduced if the system fish is cultured with other organisms, such as plants used as biofilter, which can convert nutrient discharges into valuable products. Schneider *et al.* [23] concluded that the combination of fish culture with subsequent phototrophic and herbivorous conversion increases nutrient retention in the culture system (e.g., 20%–42% feed nitrogen to 29%–45% feed nitrogen). This relative small increase is due to the herbivores, as herbivorous conversion substantially decreases the nutrient retention achieved by phototrophic conversion by 60%–85% feed nitrogen and 50%–90% feed phosphorous.

Other compounds that are present in aquaculture wastewater are feed-derived waste, antibiotics and some hormones, as described by Tacon *et al.* [33]. The feed-derived waste includes components that are either dissolved, such as phosphorus (P) and nitrogen (N) based nutrients, or that are in the solid phase such as suspended solids, as described by Losordo and Westers [21]. These solids can commonly carry 7%–32% of the total nitrogen (TN) and 30%–84% of the total phosphorus (TP) in wastewater. The remainder is transported out of the farm in the dissolved fraction, because it is largely not possible to remove them by particle separation techniques, which are commonly employed for aquaculture wastewater treatment [18].

Cripps and Kelly [24] found that the amount of SS, TN and TP were commonly low in aquaculture effluents, at about 14, 1.4 and 0.13 mg L^{-1}, respectively. However, this waste may vary depending on the aquaculture system and can cause a negative effect on the environment. Lin *et al.* [20] reported that nutrient concentrations in a fishpond increased as feed residue and fish excreta accumulated and the influent concentrations in the constructed wetlands system ranged from 0.12–14.7 mg NH_4–N L^{-1}, 0.02–1.5 mg NO_2–N L^{-1}, 0.01–5.3 mg NO_3–N L^{-1}, and 3.1–17.7 mg PO_4–P L^{-1}.

4.1.3. Feed Quality

Appropriate treatment technology and waste management must be adequate to facilitate the removal of particles as described by Cripps and Bergheim [18]. A very important issue is to improve feed quality, with a greater bio-availability of phosphorus and proteins, reducing the amount of fish excreta. Improved pellet integrity with subsequent slower breakdown rates and optimized feeding systems and protocols has also reduced wastage [18].

The development of “high-energy diets” with increased fat content, reduced carbohydrate levels, reduced protein levels, and improved digestibility has significantly decreased waste production in salmonid farming. In a standard diet for salmonids, the following fractions of the main components were reported by Åsgård and Hillestad [34] to be indigestible and excreted as fecal waste: 13% of the protein, 8% of fat, 40% of carbohydrate (fiber completely indigestible), 17% of organic matter, 50% of ashes and 23% of dry matter; about 40% of ingested protein N is excreted as dissolved N (TAN=NH_3+NH_4^+) by salmon. Recent studies indicate that a minimum of 11 g kg^{-1} dietary P is required by juvenile Atlantic salmon [35]. The daily nutrition discharges per fish (DND) for nitrogen and phosphorus are predicted by the following equation [36]:

$$DND\ \ (N,P) = nutrient\ fed - nutrient\ gain \quad (1)$$

where

$$nutrient\ fed = ration\ fed(g)\ X\ nutrient\ in\ \ feed(g\ g^{-1}\ diet) \quad (2)$$

$$nutrient\ gain = growth(g)\ X\ nutrient\ in\ \ fish(g\ g^{-1}\ \ fish) \quad (3)$$

At a feed conversion ratio (FCR) of 1.0 kg feed kg^{-1} gain, the estimated discharges from juvenile salmonids, in terms of g (N, P) kg^{-1} fish gain, are about 33 g N (26 g dissolved and 7 g solid-bound) and 7.5 g P (80%–90% solid-bound) [18]. Based on digestibility estimates of

typical diets [34], the calculated discharge of suspended solids from salmon and trout farms should be 150–200 g SS kg^{-1} fish gain at a FCR of 0.9–1.0.

As described above, it is clear that the best way to reduce the quantity of discharged waste is to improve the feed management. The required capacity of treatment systems can then be minimized, thus reducing capital and operating costs. Technology for monitoring uneaten pellets has been shown to be a useful means of reducing wastage [37]. Reduced water consumption, often by combining recirculation and addition of oxygen, is a means to improve the utilization of the water supply and to reduce the discharged effluent load because of improved treatment efficiency [38].

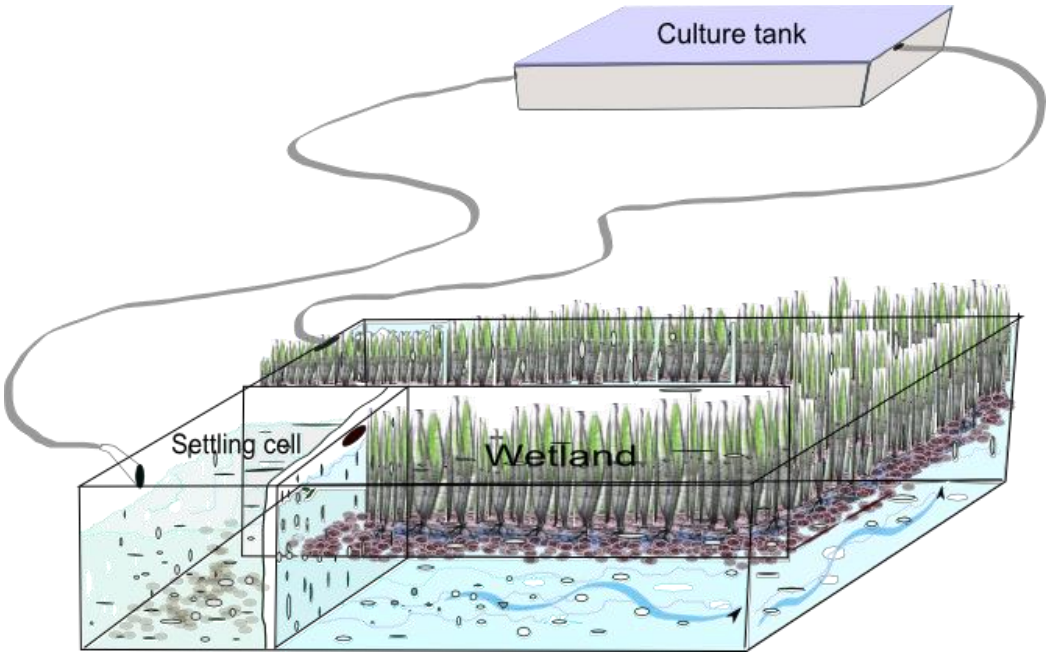
4.1.4. Bead Filters

Bead filters or expandable granular biofilters (EGBs) can operate as both mechanical and biological filters [39,40] and for this reason they have been used in recycling systems. There are several potential ways for beneficial disposal of organic waste from aquaculture: application on agriculture land, composting, vermiculture and reed drying beds [41,42]. Newly produced sludge from aquaculture is considered a good 'slow-release' fertilizer in agriculture with a high concentration of organic matter, nitrogen and phosphorus, but with a low potassium content [29,43,44].

4.1.5. Wetlands

Constructed wetland technology has grown in popularity for wastewater treatment since the early 1970s [45]. Wetlands are a well-established and cost-effective method for treating wastewater, such as municipal or domestic sewage, industrial and agricultural wastewater, landfill leachate, and stormwater runoff as described by Webb *et al.* [46] (Figure 1).

Figure 1. Typical man-made constructed wetland for a recirculation system.



Various biotic and abiotic processes regulate pollutant removal in wetlands [47,48]. Microbial mineralization and transformation (e.g., nitrification–denitrification) and uptake by vegetation are the main biotic processes, whereas abiotic processes include chemical precipitation, sedimentation, and substrate adsorption. Constructed wetlands are characterized by the advantages of moderate capital costs, low energy consumption and maintenance requirements, landscape esthetics and increased wildlife habitat [45].

Sindilariu *et al.* [49] concluded that compared to standard mechanical effluent treatment the efficiency of the sub-surface flow wetland for TSS polishing is in the range of micro-screening. Webb *et al.* [46] also demonstrated the effectiveness of wastewater treatment from land-based intensive marine aquaculture farms by constructed wetlands planted with *Salicornia* spp. Other studies [20,50–52] have demonstrated that constructed wetlands can efficiently remove the major pollutants from catfish, shrimp and milkfish pond effluents, including organic matter, SS, N, P, and phytoplankton under a low hydraulic loading rate (HLR) and long hydraulic retention time (HRT) ranging between 0.018–0.135 m day^{-1} and 1–12.8 days, respectively. These hydraulic conditions would result in a wetland size being 0.7–2.7 times the size of the pond area for treating the polluted fishpond effluents [20,50]. There are other studies where the size of wetlands varies greatly, as shown by Buhmann and Papenbrock [10]. Based on this, it is important to calculate the right size of the wetland.

4.1.6. Wetland Area Estimation

Pollutant removal in constructed wetlands, as described by Lin *et al.* [53], can be estimated by using the first-order plug flow kinetic model. This model is given as follows when omitting the background pollutant concentration [45,47]:

$$\frac{C_e}{C_i} = \exp(-kt) = \exp(-\frac{k\varepsilon h_w}{HLR}) \quad (4)$$

Where C_i = influent pollutant concentration (mg/L), C_e = effluent pollutant concentration (mg L^{-1}), t = nominal hydraulic retention time (day), k = first-order removal rate constant (day^{-1}),
HLR = hydraulic loading rate (m day^{-1}), ε = porosity of wetland, and h_w = water depth of wetland (m). The previous equation can be rearranged to provide an estimate of wetlands surface area needed for wastewater treatment:

$$A_w = \frac{Q(\ln C_i - \ln C_e)}{k\varepsilon h_w} \quad (5)$$

Where Q = flow rate of wastewater through wetlands (m^3/day).

$$Q = rA_t h_t \quad (6)$$

Where r = recirculating ratio is defined as the ratio of daily flow of recirculating water to total water in the culture tank (day^{-1}), A_t = surface area of the culture tank (m^2), h_t = water depth of culture tank (m). If Equation (6) is substituted into Equation (5), then the A_w/A_t ratio is found to be given as:

$$\frac{A_w}{A_t} = \frac{rh_1(\ln C_i - \ln C_e)}{K\varepsilon h_w} \quad (7)$$

According to Shpigel *et al.* [54], using constructed wetland (CW) systems for effluent treatment requires a relatively extensive area. About 10,000 m^2 of CW with *Salicornia* spp. are required to remove nitrogen and total suspended solids produced from 900 kg of 45% crude protein fish feed (11 m^2 kg^{-1} of feed) during one year.

4.1.7. Salt-Tolerant Plants used as Biofilters in Wetlands

The expansion of aquaculture and the recent development of more intensive land-based marine farms require commercially-valuable halophytic plants for the treatment of saline wastewater [46]. Research on wastewater treatment has been done using wetlands with halophytic plants (for a classification of plant species tolerant to different salinities see Buhmann and Papenbrock [10]). These plants have a high tolerance to salinity and may be used for absorption of nitrates, phosphates and other compounds. Halophytic plants differ in presence and specificity of aerenchyma which can influence the presence of oxygenated zones within the soil and therefore the growth of certain bacteria and processes such as ammonification and nitrification can take place [10].

Lin *et al.* [20] used a free water surface flow (FWS) wetland planted with water spinach (*Ipomoea aquatic* FORSSK.) in the front half and a native weed (*Paspalum vaginatum* Sw.) in the second half. The subsurface flow (SSF) wetland was planted with common reed (*Phragmites australis* (CAV.) TRIN. EX STEUD.). During initial plant establishment, the wetland water level was kept static, with a water depth of 5 cm for the FWS and 40 cm for the SSF. The planting densities were 12% of wetland cover for the FWS and four plants m^{-2} for the SSF. The aquatic plants grew rapidly to colonize the wetlands since influent was continuously added. Plants were not harvested during this study [20]. In 2005, Lin *et al.* [53] reported the use of Cattail (*Typha angustifolia* L.) and *P. australis* that were planted in the FWS and SSF cell, respectively. At the beginning, both cells had an initial density of around 6 plants m^{-2}, and at the end of the study, a plant density of more than 90 plants m^{-2} was observed [53].

The economic attractiveness of a halophytic biofilter can also be upgraded by the use of salt-tolerant species with a commercial value [55]. Brown *et al.* [2] studied the feasibility of wetlands equipped with different halophytes (*Suaeda esteroa* Ferren and Whitmore, *Salicornia bigelovii* Torr. and *Atriplex barclayana* (Benth.) D. Dietr.) with a potential as forage or oilseed crop as biofilter for saline aquaculture effluents.

Grieve and Suarez [56] found *Portulaca oleracea* L. to be tolerant for chloride- and sulfate-dominated salinities and to be a valuable, nutritive crop. *Plantago coronopus* L. has been reported to be a potential cash crop for human consumption. It contains valuable substances such as Vitamins A, C and K as well as calcium [57]. However, it has been shown that some plants can sequester significant amounts of antibiotics. Therefore, the question of quality control for vegetables has to be solved prior to selling such products.

Buhmann and Papenbrock [10] reported that a series of studies on the purification of recirculating aquaculture process water by constructed mangrove wetlands was conducted, resulting in a faster growth of shrimp in the treatments with water exchange to ponds planted with mangroves (*Rhizophora* spp.).

4.1.8. Removal Efficiency of Wetlands

Lin *et al.* [20] described that the average removal efficiency of a wetland system was 86%–98% for NH_4–N, >99% for NO_2–N, 82%–99% for NO_3–N, and 95%–98% for total inorganic nitrogen (TIN). These efficiencies were extremely high and were only slightly affected by the hydraulic loading rate (1.8–13.5 cm day^{-1}). In the same research, it is stated that the overall removal efficiency for phosphate decreased markedly from 71.2%–31.9% as the hydraulic loading rate increased from 2.3–13.5 cm day^{-1}. This constructed wetland system also performed well with respect to the removal of chemical oxygen demand (25%–55%), suspended solids (47%–86%) and chlorophyll a (76%–95%) from the fishpond effluent. In another research done by Lin *et al.* [53], the average removal of TSS was 66% under high hydraulic loading rates (1.57–1.95 m day^{-1}). Five-day biochemical oxygen demand (BOD_5) was, on average, removed by 37% and 54% across the FWS–SSF wetland in Phases 1 and 2, respectively. Phase 1 was conducted during a warm season from April to June and Phase 2 was performed during a cold season from August to January. Consequently, overall total ammonia nitrogen (TAN) reduction percentage of the FWS–SSF wetland averaged 66% and 64% in Phases 1 and 2, respectively. The whole treatment wetland basically showed effective NO_2-N removal with average reduction efficiency of 94% and 83% (average removal rate of 0.16 and 0.58 g m^{-2} day^{-1}) in Phases 1 and 2, respectively. In applications for wetland treatment of aquaculture wastewater and recirculating water, an efficient nitrate removal between 68% and 99% was demonstrated [20,58].

The TIN removal efficiency also depends on the nutrient load, which was demonstrated by Webb *et al.* [46]. Zachritz and Jacquez [59] and Panella *et al.* [60] concluded that wetlands can also be potentially used for treating the recycling water in a recirculating intensive aquaculture system by operating at higher hydraulic loading rates and consequently with lower removal efficiency. Nevertheless, further research on recirculating aquaculture systems is needed, focusing on higher hydraulic loading rates and their effect on fish growth and environmental effects. With regard to environmental effects, Lin *et al.* [53] concluded that the treated effluent from wetland cells can be discharged directly to the water body if a partial water exchange or draining after harvesting is necessary. Something important to consider is to keep the NO_3-N level below 1000–3000 mg L^{-1} as higher levels are considered toxic to many fish and invertebrates [22].

4.2. Present Problems

Wastewater treatments are usually physical processes, including sand and mechanical filters. Biological processes such as submerged biofilters, trickling filters, rotating biological contactors, and fluidized bed reactors are employed in the oxidation of organic matter,

nitrification, or denitrification. The disadvantages of these treatment methods are that they produce sludge, require much higher energy and depend on frequent maintenance. The development of an effective, low-cost treatment is therefore imperative if aquaculture is to expand continually at the present rate [59]. Constructed wetland systems are characterized by the advantage of a high effectiveness in the treatment of wastewater, but the disadvantage is that they require a considerable area of land, being 0.7–2.7 times the size of the pond area. Thus, wetland methods may need a large land area when a great amount of aquaculture wastewater needs to be treated. For this reason, there is a concern about the feasibility of wetlands as a cost effective method because wetlands typically require a low hydraulic loading rate and a long hydraulic retention time to achieve efficient pollutant removal [53]. Nevertheless, Sindilariu *et al.* [61] pointed out that the combination of effective pre-treatment (80% TSS removal) with small constructed wetlands processing high hydraulic loads, are economically most feasible, with annual costs of €15,450. For a 100 L s^{-1} trout farm with an annual production of 770 kg (L s^{-1})$^{-1}$, this represents a production cost increase of €0.20 kg^{-1}.

5. What can be Learned for the Future? Facts and Aspirations

A large body of good-quality research has been carried out worldwide on different integrated aquaculture systems that use plants to take up waste nutrients and, at the same time, add to the income of the farms. Research over three decades has brought the integrated land-based technology to a commercial reality. Through plant biofilters, often in combination with additional filtering species, integrated aquaculture recycles nutrients into profitable products while restoring water quality. Fish–phytoplankton–shellfish systems convert the fish waste into bivalves, which have a large global market value. Fish–seaweed–macroalgivore (such as abalone and sea urchin) systems have a choice of marketing either the seaweed or the macroalgivore, while they use less land than the fish–phytoplankton–shellfish systems and maintain a more stable water quality. Integrated aquaculture, in both freshwater and seawater, can be profitable, thanks to the sales of the biofilter organisms such as vegetables, shellfish and seaweed. The results are higher yields and income per ton of feed and per ton of water. Furthermore, the integrated culture system fulfills, at no extra effort, practically all the requirements of organic aquaculture, a feature that opens up new lucrative markets to the aquaculturist [11].

The development of new finfish species is a high priority for the diversification of the aquaculture in several countries, in order to expand the production to high-value resources and different geographical zones. Cultivation in RAS for the whole life cycle is currently being established. Modern closed RAS can operate with artificial seawater and less than 1% of water renewal per day. These high-tech systems allow the land-based cultivation of ‘exotic’ species of high commercial interest, close to the consumer, and with zero discharge of nutrients and organic matter into natural ecosystems when combining with IMTA. Such systems offer the necessary bio-security for the culture of non-native species, water quality control as well as waste management. Biosecure RAS also avoid disease outbreaks and parasites due to the lack

of intermediate hosts. Additionally, product traceability is possible. This type of technology is environmentally sound and contributes to the sustainability of aquatic food production [15].

The environmental sustainability of modern RAS does not rely on production results and/or good water parameters only, but also on the optimization of the use of land, energy, feed and water. Recent developments of IMTA systems allow the use of RAS waste products as nutrients, coupling different water loops with the main fish production water system. Another possibility is the implementation of end of pipe treatments such as artificial wetlands. A deeper understanding of the interaction between nutrient inputs (feed), nutrient retention (growth) and outputs (soluble and particulate waste) will help address the sustainability of RAS and integrated land based aquaculture [15].

Good practice in the management of water resources will aim to diminish the cost of water, reducing consumption and maximizing the reuse or recycling of supply water, while returning it to the natural waters with acceptable physicochemical and biological characteristics and, hence, avoiding negative impacts on ecosystems. In this context, there has been a shift towards community integration of aquaponics that offers job opportunities and training while growing food for the community as can be found in several countries (USA, Israel, Germany, The Netherlands) [54]. Taking into consideration that the future development of marine aquaculture will face a paradigm shift, it is important that a modern medium-scale (500 mt y^{-1}) urban RAS is able to deliver high quality fish and other aquaculture products to niche markets in areas with high population density [15]. In addition, aquaponic gardeners from all around the world have gathered on online community sites and forums to share their experiences and promote the development of this form of gardening. Recently, aquaponics has been moving towards indoor production systems. Entrepreneurs are utilizing vertical designs to grow food all year round [62].

6. The Potential Use of *Salicornia* spp. in Aquaponics

The limited resources of freshwater for agriculture, aquaculture and the ongoing increase in soil salinity throughout the world demands the development of new crops that are able to tolerate higher salt concentrations than conventional agricultural crops [63,64]. Different species of *Salicornia* have been studied recently, demonstrating their high potential as new salt-tolerant crop plants based on their tolerance to high salt concentrations up to seawater concentration, and potential use for food, fodder, acting as biofilter for treating wastewater, oil production, gas production and other industrial uses (Figure 2).

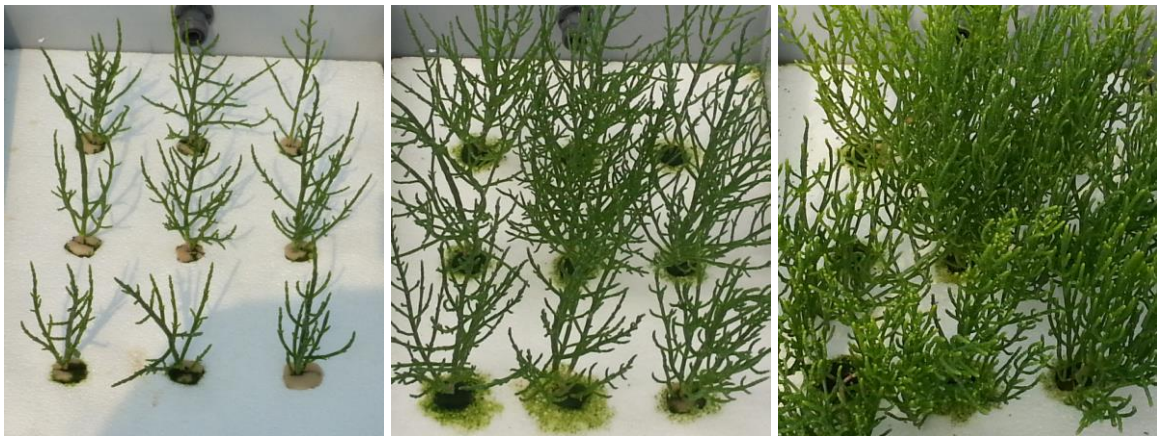
It was experimentally shown that *Salicornia* spp. has a great potential for extracting inorganic contaminants from wastewater, such as nitrates and phosphates. As Buhmann and Papenbrock [10] pointed out, there are currently various approaches to tap the market for halophytes especially for *Salicornia* spp. as vegetable as well as using halophytes as biofilter and valuable side product for aquaculture wastewater treatment in temperate and subtropical regions.

In the same review, Buhmann and Papenbrock [10] stated that the halophytic plants recycle the nutrients generated in a fish culture in terms of biomass production and contribute to

maintain appropriate quality in the process water of the recirculating aquaculture system. One of the main problems using plants as biofilters is that after their useful life, their high salt-containing biomass is discarded and can contaminate other resources. To permanently remove the nutrients taken up by plants and to no longer return them to the water bodies, it is important to harvest them frequently and use the biomass for food and fodder and think about meaningful applications for the rest of the plants. The income generated from selling *Salicornia* spp. as an agricultural crop, together with savings on water treatment and potential fines, contributes to the system's economical viability as described by Shpigel *et al.* [54]. Especially, the cultivation of *Salicornia* spp. in aquaponic systems shows many advantages over sand or soil cultures, such as controllability, reproducible mass cultures of high numbers, hygienic aspects *etc.* (Figure 2).

As *Salicornia* spp. is a new fresh vegetable for human consumption, product quality is a major concern. *Salicornia* spp. shoots are not only a good source of minerals, but they also contain proteins, various vitamins [65] and higher total lipid and omega-3 contents than spinach, lettuce and mustard green leaves [66]. Thus, *Salicornia* ecotypes may attract considerable interest as an alternative source of polyunsaturated fatty acids for human consumption, even when grown on full-strength seawater [67].

Figure 2. One of the promising *Salicornia* species in hydroponic culture is *Salicornia dolychostachya* Moss. Photos: Christian Boestfleisch, Institute of Botany, Hannover.



As a new crop plant, several growing conditions and selection of genotypes need to be optimized before commercial success is guaranteed [67,68]. With respect to growing conditions, the nutritional content of *Salicornia* can vary depending on salinity. Yousif *et al.* [69] reported a decline in the content of K^+, Ca^{2+} and Mg^{2+} cations with increasing Na^+ availability which has been noted for both halophytes and non-halophytic plants, while augmented Cl^- contents are believed to have antagonistic effects on NO_3^- uptake. Nevertheless, Ventura *et al.* [67] reported no changes in these elements with high salt concentrations, with the following effects on ion concentrations in the shoots: no change in Ca^{2+} and Mg^{2+}, a slight increase in K^+, and marked elevations in Na^+ and Cl^-. Total polyphenol, β-carotene and ureides, all known for their antioxidant capacities, rose with increasing seawater percentage, which indicated improved nutritional values for *Salicornia* spp. irrigated with high concentrations of seawater. These plants have high total shoot lipid contents of up to 2.41 mg g^{-1} fresh weight, which includes an omega-3 fraction of 47.6% of the total fatty acid content. Therefore, the high fatty acid content of the annual *Salicornia* spp. was not significantly affected by increasing salt concentrations [67].

Biogas production can be another important use of the *Salicornia* spp. biomass. The preservation of the environment and the increasing consumption of energy resources are two important aspects, requiring the application of new low-cost technologies for the reuse of waste, conducive to obtaining other useful products such as biogas. Today, the search for renewable energy sources is a challenge for humanity. Worldwide, the use of renewable energy sources is indispensable for development which ensures not only the production of fuel, but in many cases, eliminating waste pollutants that harm the environment. From this point of view, even high salt-containing *Salicornia* spp. biomass can be used for biogas production, through an anaerobic process after optimization. To date, there are no data and experiences on this topic, which is one of the research activities carried out at the Institute of Botany, Leibniz University Hannover, Germany.

7. The Potential Use of Mangroves

For tropical regions, the use of plants as biofilter is also promising. Actually, many aquacultural ponds have been constructed in previous mangrove areas. After the recent strong Tsunami events, it has been more and more realized how important mangroves are for the protection and stability of the coastlines. Mangroves also promote biodiversity because their roots provide shelter for fish, mammals and invertebrates and they have a high economic and ecological value because they act as fishponds. Fish growth is conducted under their roots, so these plants are fundamental to ensure the sustainability of the fishing industry (Figure 3). At the same time, mangroves contribute to nutrient retention, protection and stabilization of shorelines, preserving water quality, climatic regulation and erosion prevention. Mangroves are being widely used to treat wastewater and simultaneously can give protection against natural disasters. Coastal wetlands, such as reefs, marshes and mangroves, act as first-line defenses against the potential devastation through tsunamis and storm events. Mangrove forests occupy 14,650,000 ha of coastline globally [70], with an economic value on the order of 200,000–

900,000 USD ha^{-1} [71]. Regardless of their monetary value, mangrove ecosystems are important habitats, especially in developing countries, and play a key role in human sustainability and livelihoods [72], being heavily used traditionally for food, timber, fuel, and medicine [73].

Figure 3. Mangrove forest in Tamil Nadu, India. Mangrove roots provide a tangled underwater habitat for many marine species. Photo: Jutta Papenbrock, Institute of Botany, Hannover.

Mangrove forests can attenuate wave energy, preventing the damage caused by tsunamis, as shown by various modeling and mathematical studies [74–79] which indicate that the magnitude of absorbed energy strongly depends on forest density, diameter of stems and roots, forest floor slope, bathymetry, the spectral characteristics (height, period, *etc.*) of the incident waves, and the tidal stage at which the wave enters the forest. For instance, one model estimates that at high tide in a *Rhizophora*-dominated forest, there is a 50% decline in wave energy by 150 m into the forest [74]. Moreover, Mazda *et al.* [77] highlighted that the thickly grown mangrove leaves effectively dissipate high wave energy which occurs during storms such as typhoons and, therefore, protect coastal areas.

Especially in rural tropical areas, aquacultures provide a source of income and employment opportunities and therefore aid economic and social development. A very attractive idea is to combine all positive effects of mangroves to make aquaculture in the tropics more environmentally friendly. Vegetation could be used to filter waste water from aquaculture and additionally provide biodiversity, coastal protection and economic services to the community. First trials have been successfully conducted, for example in the Philippines [80]. However, more research is necessary and the idea needs to be promoted in a more professional way in several tropical countries.

8. Outlook

In order to feed the growing population in the world with well-balanced food of sufficient quality, the development of sustainable aquacultural systems is fundamental. Several ways to improve the systems currently used are described and discussed in this review. Optimized RAS combined with biofiltering organisms such as plants or algae seem to be the most promising way. Actually, due to their highly flexible metabolism, higher plants might be even more suitable to act as biofilters than algae. Plants have evolved sophisticated detoxification systems against several xenobiotics following the uptake. Different plant species might be able to degrade and/or detoxify hormones and antibiotics sometimes used in aquaculture. The plants might reduce toxicity and sequester the xenobiotics in phytotransformation. This could also be a very important aspect in using plants as biofiltering organisms for the future.

Acknowledgements

Financial support of Ariel Turcios by the DAAD and the Universidad de San Carlos de Guatemala is gratefully acknowledged. We would like to thank Maike Paul, Hannover, for correcting the English language. We acknowledge the support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of Leibniz Universität Hannover.

Author Contributions

The basic structure of this paper was suggested by Jutta Papenbrock. In all other aspects both authors contributed equally to this article.

Conflicts of Interest

The authors declare no conflict of interest.

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Chapter 3

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *Tripolium pannonicum*, Biomass & Bioenergy. 87: 69-77. doi:10.1016/j.biombioe.2016.01.013

Biomass and Bioenergy 87 (2016) 69–77

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *Tripolium pannonicum*

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ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form
10 January 2016
Accepted 22 January 2016
Available online xxx

Keywords:
Anaerobic digestion
Halophyte plants
Microbial adaptation
Renewable energy
Sodium inhibition
Tripolium pannonicum

ABSTRACT

The halophyte species Sea Aster (*Tripolium pannonicum*) was grown with different concentrations of artificial seawater. In a second experiment, *T. pannonicum* was cultivated with a nutrient solution containing different concentrations of NaCl. This halophyte biomass was used to determine the biogas production potential. According to the findings, it is possible to produce high yields of methane using biomass from halophytes cultivated in the presence of salt. Biogas and methane yield are influenced by the salt content of the plant tissue, however, high concentrations of salt in the anaerobic reactors itself inhibit the biogas and methane production. The highest methane yield is obtained using plant substrates grown at 22.5 g L^{-1} sea-salt with a value of 313 cm^3 g^{-1} of VS. When treating *T. pannonicum* with different concentrations of NaCl, biogas and methane yields are highest when using plant substrates grown at 30 g L^{-1} to produce values of 554 cm^3 g^{-1} of VS and 447 cm^3 g^{-1} of VS, respectively. Other research was carried out to study the effect of sodium on the biogas and methane yields using substrate from *T. pannonicum* cultured under non-saline conditions and adding different amounts of NaCl to the anaerobic reactors. Adding NaCl to the reactors decreases the biogas and methane production but using a salt-adapted inoculum increases the biogas yield in comparison to the non-adapted inoculum.

1. Introduction

Renewable energy technologies are clean sources of energy that have a much lower environmental impact than conventional energy technologies. Biogas production from agricultural biomass or other sources of organic waste through the process of anaerobic digestion is of increasing importance. Anaerobic digestion is widely applied to treat various residues while simultaneously delivering renewable energy that substitute for fossil fuels. Several plant species have been tested for biogas production such as maize, sunflower, grass, and clover, and are listed in the German positive list as renewable plant species [1], but there is still very little work done on halophytic plant species. These halophytes are able to grow in saline soils and uptake significant amounts of salt. Therefore, they can be used for remediation for saline lands. With the declining availability of arable land and water resources, these halophytes are an alternative which can be grown in coastal areas, and the produced biomass could be used for food, fodder or biogas production in the sector of renewable energy. In addition, halophytes can be used in constructed wetlands in order to reduce some chemical and biological compounds, due to the fact that wetlands have proven to be well-established and a cost-effective method for treating wastewater [2,3].

As mentioned above, many kinds of substrates have been used for biogas production. For example, Amon et al. [4] reported that methane yields from late ripening maize varieties range between 312 cm^3 g^{-1} of VS and 365 cm^3 g^{-1} of VS (milk ripeness) and from 268 cm^3 g^{-1} of VS to 286 cm^3 g^{-1} of VS (full ripeness). According to Amon et al. [4] maximum methane yield per hectare from late ripening maize varieties range between 7.1 dam^3 ha^{-1} and 9.0 dam^3 ha^{-1}, early and medium ripening varieties yield from 5.3 dam^3 ha^{-1} to 8.5 dam^3 ha^{-1} when grown in favorable regions. Amon et al. [5] found that methane yields of cereals range from 3.2 dam^3 ha^{-1} to 4.5 dam^3 ha^{-1}, sunflowers from 2.6 dam^3 ha^{-1} to 4.55 dam^3 ha^{-1} and alpine grassland between 2.7 dam^3 ha^{-1} and 3.5 dam^3 ha^{-1}.

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http://dx.doi.org/10.1016/j.biombioe.2016.01.013
0961-9534/

The biogas yield also depends on some chemical constituents that can influence the methanogenesis process such as the salt content in the substrate [6]. High salt content causes microbial cells to dehydrate due to osmotic pressure [6] and [7], where the toxicity is predominantly determined by the cation [8] and [9]. Halophytes tend to accumulate salt in their tissues, for this reason it is important to determine the salt concentration in the material used for anaerobic digestion. In addition, salinity tends to change the element content in the plant tissues and some organic compounds such as crude lipids (CL), hemicellulose (HCEL), acid detergent lignin (ADL) and total sugars (CH). According to Rath et al. [10], CL, HCEL, ADL, and CH have an influence on biogas yield, with the first two biochemical constituents being positively correlated with the biogas production and the last two showing a negative relationship. In line with this, Dandikas et al. [11] concluded that based on the fodder analysis of different energy crops, it is revealed that the biogas yield is significantly negatively correlated with ADL and positively correlated with HCEL. However, Chen et al. [8] reported that also some elements can enhance or inhibit the methane production, such as Na, Ca, K and Mg. The C/N mass ratio in the anaerobic process may influence the pH, free ammonia, total ammonium, and therefore, the methane yield as well [8].

To date, there have been only limited systematic studies on the effect of salt on the fermentation process as well as the potential use of halophytes for biogas production; therefore, the main aim of this research is to study the inhibition effect salt has on biogas and methane production using halophyte biomass. Other aims of this research are: to characterize the substrate and the inoculum used in the anaerobic process, to quantify the biogas and methane yields with varying concentrations of salt in the anaerobic reactors, to determine whether the plant material with high content of salt can be used as a substrate for biogas production and to study the microbial adaptation to the salt by the reuse of inoculum containing different salt concentration.

2. Material and methods

2.1. Plant biomass for biogas

The seeds of *Tripolium pannonicum* (Jacq.) Dobrocz. were collected at the North Sea, Germany (53°29′13″N; 8°03′16″E). The agronomic handling from sowing through transplanting was carried out as described by Buhmann et al. [3]. From February 2014 to April 2014, *T. pannonicum* plants were grown under different artificial seawater concentrations (0, 15, 22.5, and 30 g L^{-1}) (hereinafter called "E1"). In another experiment carried out from July to September 2014, *T. pannonicum* was grown under different NaCl concentrations (0, 15, 30 and 45 g L^{-1}) (hereinafter called "E2"). All the plants were grown for five weeks after transplanting in hydroponic conditions. These experiments were conducted in a greenhouse at the Institute of Botany, Leibniz University Hannover, Germany (52°23′42″N; 9°42′13″E), with temperatures between 15 °C and 29 °C. Plants were exposed to 12 h of artificial light (sodium vapor lamps, SON-T Agro 400, Philips). Light intensity ranged from 65 μmol m^{-2} s^{-1} to 850 μmol m^{-2} s^{-1} depending on the time of the year, the time of the day and the weather conditions. Polypropylene containers (400 mm × 300 mm × 175 mm) with a capacity of 16 L were used. For the plants cultured with artificial seawater (salt mixture from Seequasal GmbH, Münster, Germany), each container had 13.5 L solution containing 303 mg L^{-1} $NaNO_3$, 44 mg L^{-1} $H_2NaPO_4xH_2O$ and 0.56 mg L^{-1} Fe-EDDHA (Duchefa, Haarlem, Netherlands). For the plants grown with pure NaCl (BioChemica, AppliChem GmbH, Germany), each container had 13.5 L solution containing 303 mg L^{-1} KNO_3, 472 mg L^{-1} $Ca(NO_3)_2x4H_2O$, 115 mg L^{-1} $NH_4H_2PO_4$, 123 mg L^{-1} $MgSO_4x7H_2O$, 1.865 mg L^{-1} KCl, 0.775 mg L^{-1} H_3BO_3, 0.17 mg L^{-1} $MnSO_4xH_2O$, 0.29 mg L^{-1} $ZnSO_4x7H_2O$, 0.06 mg L^{-1} $CuSO_4x5H_2O$, 0.06 mg L^{-1} $MoNa_2O_4x2H_2O$, and 0.28 mg L^{-1} Fe-EDDHA. The water was constantly aerated by small compressors and one air stone in the middle of each tank (Eheim, Deizisau, Germany). The hypocotyl was fixed with soft foam in 35 mm holes. The water level was adjusted constantly in each tank with tap water to compensate for evapotranspiration and therefore the salinity was kept constant. Salinity and nutrient concentration were monitored weekly. In E2, the culture medium was changed every two weeks. Each experimental unit consisted of eight plants per container, with three replicates per treatment. After harvesting, the aerial part of the plants was frozen immediately in liquid nitrogen and stored at −80 °C. Before using the plant material as substrate for biogas production, it was homogenized according to the VDI 4630 protocol [12].

2.2. Salinity in plant tissue

There are different methods to measure the electrical conductivity in plant tissue, for example through ground plant tissue extracts using a conductivity meter [13,14], and [15]. To determine the conductivity in the entire plant tissue, three different ways were applied: boiling the plant material in deionized water, grinding the fresh material and afterwards make a dilution in deionized water, and diluting ashes in deionized water. The conductivity in the solution was determined in Practical Salinity (PS) by a pH meter (Multi 350i pH/ISE/DO/conductivity measuring instrument, Wissenschaftlich-Technische Werkstätten GmbH, Germany).

2.3. Biomass compositional analysis

The dry plant material of *T. pannonicum* was obtained by cultivating plants with different NaCl concentrations (0, 15, and 30 g L^{-1}), harvesting and drying the plant material before grinding the plant material with Planetary Ball Mill PM 100 (Retsch GmbH, Germany) to fine powder with a centrifugal force of 15.96× *g* for 6 min. Then 50 g–100 g per sample were packed into a plastic container and shipped to Landwirtschaftliche Untersuchungs-und Forschungsanstalt (LUFA) Nord-West, Oldenburg, Germany, for the determination of crude ash (CA), crude protein (CP), crude lipid (CL), total sugars (CH), crude fiber (CF), Acid Detergent Fiber (ADF om), Neutral Detergent Fiber (NDF om) and Acid Detergent Lignin (ADL). CA, CP, CL, CH, and CF were analyzed according to the Commission Regulation [16], and ADF om, NDF om, and ADL according to the VDLUFA Bd. III. Carbon and nitrogen were determined using a C-N-S elemental analyser (Vario EL III, Elementar Analysensysteme, Hanau, Germany).

2.4. Biogas test

The procedure for the biogas test was conducted according to the VDI 4630 protocol [12]. Four experiments were carried out from February 2014 to February 2015. The first experiment was conducted using fresh plant material from *T. pannonicum* as substrate, cultivated under different artificial sea-salt concentrations (0, 15, 22.5, and 30 g L^{-1}) (E1). The second experiment was conducted using *T. pannonicum* as substrate, grown under different NaCl concentrations (0, 15, 30, and 45 g L^{-1}) (E2). The third experiment was carried out using *T. pannonicum* as substrate cultivated under non-saline conditions. In this experiment, different amounts of NaCl were added to the anaerobic reactors (0, 224, 450, 636, and 887 mg per reactor). Each reactor contained 200 cm^3 of seed sludge. The total concentration of sodium per liter of anaerobic medium (substrate + inoculum) was determined by summing the sodium

content in the plant material, in the seed sludge and the added quantity, to obtain a final concentration per treatment of (929, 1370, 1816, 2181, and 2675 mg L^{-1}). To investigate the microbial adaptation to the salt, a further experiment was conducted reusing the inoculum from the third experiment containing different concentrations of NaCl. In this experiment, a bifactorial experiment in a completely randomized design was used. One factor consisted of four levels with different sodium concentrations in the reused inoculum (929, 1370, 1816 and 2181 mg L^{-1}), the other factor consisted of two levels, in one level no salt was added and in the other one 6.36 g L^{-1} NaCl (2500 mg L^{-1} of Na^{+}) was added to the anaerobic reactors.

The dry matter (DM) and volatile solid (VS) contents were determined for both the substrate and the inoculum (seed sludge). The dry matter was determined according to DIN EN 12880 [17]. The VS contents were determined according to DIN EN 12879 [18].

The seed sludge as inoculum was taken from the digester of the Sewage Treatment Plant, Hannover-Herrenhausen, Hannover, Germany. The fresh substrate mass fraction was 0.333 in the total mixture of substrate and inoculum. Three replicates were used for each sample (inoculum + substrate) and three replicates as blanks (containing only inoculum). All bottles were filled with the same amount of inoculum. For each substrate, the calculated amount was added to each one of the three replicate reactors.

The reactors were flushed with nitrogen for approximately 1 min to promote anaerobic conditions and after were tightly closed and incubated at 37 °C. The tests were conducted using handheld devices, taking daily pressure readings into the reactors with a manometer. Each test was terminated when the daily biogas rate was below 0.5% of the total biogas produced up to that time. After finishing the experiment, a gas sample was taken for gas chromatography analysis to determine the fraction of methane in the raw biogas samples. The biogas and methane volumes were calculated in accordance to the Gay-Lussac law and converted to volume of gas under normal conditions (273.15 K and 101.325 kPa). The dissolved biogas into the liquid phase was quantified and calculated following the Henry's law, respectively.

2.5. Statistical analysis

All statistical analyses were conducted using R, version 3.1.1 [19] and InfoStat software, version 2014e [20]. The Tukey multiple comparison test with $\alpha = 0.05$ was done to determine which means differ from the rest.

3. Results and discussion

3.1. Biomass compositional analysis

Based on this study, CA, CL, and HCEL have a positive relationship with the salt concentration in the culture medium in which the plants were grown. CP, CF, ADF, NDF, ADL, and cellulose have an inverse relationship with the salt concentration in the culture medium. Salt has not a notable influence on CH (Table 1). These organic constituents influence the biogas production, being CL and HCEL positively correlated with the biogas yield while ADL shows a negative relationship [10] and [11]. Therefore, the substrate of the plants cultured at 30 g L^{-1} NaCl has a higher potential for biogas production due to the greater amount of CL and HCEL and lower content of ADL. Chen et al. [8] reported that other elements can increase or decrease the methane production, such as Na, Ca, K and Mg. Also the C/N ratio in the anaerobic process may influence the pH, free ammonia, total ammonium, and therefore, the methane yield as well. Anaerobic digestion requires a C/N ratio between 10 and 30 [21]. However, Kwietniewska and Tys [22] reported that the optimal C/N ratio for anaerobic degradation of organic waste is 20–35. According to the results obtained in this study, the C/N ratio of the plants cultivated with artificial seawater is not significantly influenced by salinity ranging from 9.80 to 12.45, but in the plants cultivated with different NaCl concentrations, the C/N ratio is significantly changed from 6.8 at 15 g L^{-1} NaCl to 10.0 at 30 g L^{-1} NaCl with a p-value of 0.033.

3.2. Salinity in plant tissue

According to the results, sodium content and electrical conductivity in plant tissue show a direct relationship: the more sodium is abundant, the higher the electrical conductivity. The electrical conductivity in the plant tissue using ground material or ashes is quite similar. The ashes diluted in deionized water have the highest conductivity value because the plant material is completely destroyed after being incinerated; therefore, this method is more accurate to measure the electrical conductivity in the entire plant tissue. The conductivity in the tissues after boiling the plant material in deionized water is lower than determined by the other methods. Sodium content in the plant tissue from the plants grown under non-saline conditions is 4.55 mg g^{-1} FW and the electrical conductivity in the plant tissue using ashes diluted in deionized water is 11.3 practical salinity (PS), while in the tissue of the plants cultivated with a concentration of 30 g L^{-1} sea-salt shows a sodium content of 10.9 mg g^{-1} FW and an electrical conductivity of 38.1 PS (Fig. 1). This underlines that the halophyte *T. pannonicum* accumulates salt in its tissues and a high salt content in the plant material has an influence on methanogenesis [6] with sodium as the main inhibiting element [8].

3.3. Element content in the seed sludge used for biogas production

In this experiment, the element content in the inoculum used for the batch tests was analyzed using ICP–OES before starting the anaerobic digestion process. The substrate used with this inoculum was also plant material of *T. pannonicum* and cultivated with different concentrations of NaCl (E2). Ca, P, Fe, Na and K have been found at concentrations of 1147, 1108, 932, 437 and 256 mg L^{-1}, respectively. The concentration of S is 185 mg L^{-1}, while Co, Cu, Mg,

Table 1
Biomass quality composition of *T. pannonicum* cultivated with different NaCl concentrations in the culture medium (0, 15 and 30) g·L^{-1} NaCl.

	NaCl [g·L^{-1}]	CA	CP	CL	CH	CF	ADF om	NDF om	ADL	HCEL	CEL
T. pannonicum	0	19.2	22.2	2.9	7.5	11	24.9	30.6	18.2	5.7	6.7
	15	31.2	22.5	3.4	4.1	8	18.9	26.9	17.1	8	1.8
	30	33.7	19.5	3.3	6.9	6.7	14.3	23.8	12.1	9.5	2.2
Z. mays[a]			6.4	2.4	5.6	18.7	23.6	37.2	1.8	13.7	21.7

NaCl [g·L^{-1}] = gram per liter of sodium chloride in the culture medium. Data are on dry matter basis in g/100 g DM; CA = Crude Ash; CP = Crude Protein; CL = Crude Lipid; CH = Total Sugars; CF = Crude Fiber; ADF om = Acid Detergent Fiber; NDF om = Neutral Detergent Fiber; ADL = Acid Detergent Lignin; HCEL = hemicellulose; CEL = Cellulose.
[a] Data in Ref. [10].

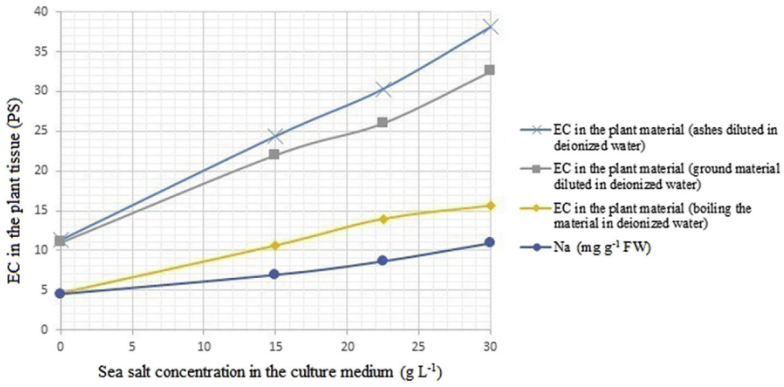

Fig. 1. Electrical conductivity (EC) in the tissues of *T. pannonicum* cultivated with different concentrations of sea-salt (in gram per liter).

Mn, Ni, and Zn remain at lower concentrations with values of (0.13, 13.00, 34.70, 9.00, 0.80, 26.90 mg L^{-1}), respectively. It has been reported that a concentration of Ca^{2+} between 2.5 g L^{-1} and 4.0 g L^{-1} in the anaerobic medium causes a moderate inhibition on methanisation of acetate, while a concentration of 200 mg L^{-1} is optimal for methanisation. A Mg^{2+} concentration of 720 mg L^{-1} and a concentration of 400 mg L^{-1} of K^{+} were reported to cause an enhancement in performance in both the thermophilic and mesophilic ranges. The optimal range of sodium to produce the highest amounts of biogas is between 230 mg L^{-1} and 350 mg L^{-1} of Na^{+} [8]. According to these findings, the content of elements in the inoculum, in theory, should not cause an inhibition on the methane yield. However, some synergistic/antagonistic effects may occur. For example, the combination of sodium and potassium, sodium and magnesium or sodium and calcium may increase the methane yield.

3.4. Biogas yield from T. pannonicum

In E1, the highest production of biogas is observed with the substrate of plants grown under non-saline conditions (control) with a value of 475 cm^3 g^{-1} of VS (p-value 0.0003). The highest specific methane yield is obtained using the substrate of the plants grown at 22.5 g L^{-1} and 15 g L^{-1} of sea-salt with a value of 313 cm^3 g^{-1} of VS and 309 cm^3 g^{-1} of VS, respectively, and a p-value <0.0001 (Table 2 and Fig. 2a). Using the substrate of E2, the specific biogas yield and the specific methane yield are higher using the substrate of the plants grown at 30 g L^{-1} NaCl with a mean value of 554 cm^3 g^{-1} of VS and 347 cm^3 g^{-1} of VS, respectively (Table 3 and Fig. 2b). These yields are similar to those obtained from other crops (Fig. 3). In terms of energy, it is more important to determine the methane yield; therefore, for E1 the most promising treatments are the plants grown at 15 g L^{-1} and 22.5 g L^{-1} sea-salt and the best treatment for E2 is at 30 g L^{-1} NaCl (Fig. 2). In addition, the biomass yield is higher at 15 g L^{-1} for the plants cultivated with artificial seawater (E1). Therefore, the biogas and methane yield per area are even higher at this salt concentration with a value of 51.9 L m^{-2} and 36.0 L m^{-2}, respectively. Regarding the plants cultivated with NaCl (E2), the greatest fresh biomass yield is obtained from the control (non-saline conditions); consequently, the biogas and methane productivities are higher using the plants cultured without NaCl with a mean value of 217 L m^{-2} and 142 L m^{-2}, respectively. In terms of kilowatt-hour per square meter of crop production, in the case of E1, the best treatment is for the plants grown at 15 g L^{-1} sea-salt with a value of 0.36 kW h m^{-2} (37.5 MW h ha^{-1} a^{-1}) and a p-value <0.0001. For E2, the best treatment is the control with a mean value of 1.42 kW h m^{-2} (148 MW h ha^{-1} a^{-1}). Seppälä et al. [25] reported that the methane and energy yields from different grass species range from 1200 m^3 ha^{-1} a^{-1} to 3600 m^3 ha^{-1} a^{-1}, corresponding from 12 MW h ha^{-1} a^{-1} to 36 MW h ha^{-1} a^{-1}. In comparison with these data, *T. pannonicum* has a high potential for biogas and methane production. In addition, this halophyte has other benefits: it can be cultivated in saline soils and be irrigated with saltwater or even with seawater and has also a potential use as biofilter for treating wastewater [3]. Moreover, using *T. pannonicum* instead of other food crops such as maize the growing competition between food and energy crops will be

Table 2
Salinity and biogas production using *T. pannonicum* as substrate cultivated with different sea salt concentrations -E1-.

Variables	Salinity treatment [g·L^{-1}]				p-value
	0	15	22.5	30	
EC in the plant tissue (PS) ± SE	11.33 ± 0.24 D	24.40 ± 0.35 C	30.33 ± 1.38 B	38.13 ± 0.79 A	<0.0001
EC in the digestate (PS) ± SE	5.57 ± 0.07 D	6.67 ± 0.12 C	7.23 ± 0.03 B	7.83 ± 0.09 A	<0.0001
Na^{+}-digestate (mg·L^{-1}) ± SE	455.2 ± 31.2 C	724 ± 27 B	941 ± 39 A	1058 ± 67 A	0.0001
Biogas ($cm^3 \cdot g^{-1}$) ± SE	475.8 ± 1.9 A	445.9 ± 3.6 B	444.1 ± 3.8 B	429.8 ± 5.8 B	0.0003
Biogas ($L \cdot m^{-2}$) ± SE	29.4 ± 3.9 BC	51.9 ± 1.3 A	33.7 ± 1.2 B	20.0 ± 1.9 C	0.0001
Methane ($cm^3 \cdot g^{-1}$) ± SE	296.5 ± 1.2 B	309.4 ± 2.5 A	313.0 ± 2.7 A	270.9 ± 3.6 C	<0.0001
Methane ($L \cdot m^{-2}$) ± SE	18.3 ± 2.5 BC	36.02 ± 0.87 A	23.72 ± 0.83 B	12.6 ± 1.2 C	<0.0001
kWh·m^{-2} ± SE	0.18 ± 0.02 BC	0.36 ± 0.01 A	0.24 ± 0.01 B	0.13 ± 0.01 C	<0.0001

EC = electrical conductivity in Practical Salinity Scale (PS); $cm^3 \cdot g^{-1}$ = cubic centimeter per gram of volatile solids; $L \cdot m^{-2}$ = liter per square meter of crop production; kWh·m^{-2} = kilowatt-hour per square meter of crop production. 1 m^3 CH_4 ≈ 10 kWh. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ≤ 0.05) between salinity treatments are indicated by different letters (A, B, C, D, within the same row).

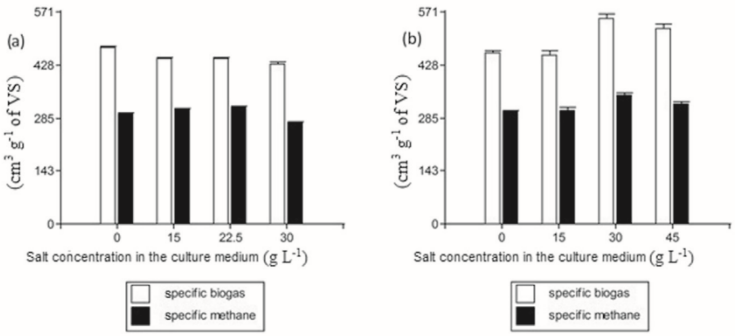

Fig. 2. Specific biogas and specific methane production. (a) Substrate of *T. pannonicum* cultivated under different artificial seawater concentrations (in gram per liter). (b) Substrate of *T. pannonicum* cultivated under different NaCl concentrations (in gram per liter).

Table 3
Electrical conductivity and biogas production using *T. pannonicum* as substrate cultivated under different NaCl concentrations -E2-.

Variables	Salinity treatment [g·L^{-1} NaCl]				*p*-value
	0	15	30	45	
EC in the plant tissue (PS) ± SE	7.87 ± 0.07 D	22.07 ± 0.64 C	35.87 ± 0.64 B	44.83 ± 0.43 A	<0.0001
EC in the digestate (PS) ± SE	6.73 ± 0.03 C	8.63 ± 0.03 B	9.80 ± 0.15 A	9.90 ± 0.06 A	<0.0001
Total Na^+ digestate (mg·L^{-1}) ± SE	930 ± 40 B	1870 ± 160 A	1988 ± 75 A	2190 ± 140 A	0.0003
Biogas (cm^3·g^{-1}) ± SE	461.1 ± 6.0 B	454 ± 10 B	554 ± 11 A	527 ± 10 A	0.0002
Biogas (L·m^{-2}) ± SE	217.7 ± 2.8 A	132.0 ± 3.0 B	41.75 ± 0.81 C	15.34 ± 0.29 D	<0.0001
Methane (cm^3·g^{-1}) ± SE	301.0 ± 3.9 B	306.1 ± 7.0 B	347.2 ± 6.7 A	323.8 ± 6.2 AB	0.0026
Methane (L·m^{-2}) ± SE	142.1 ± 1.9 A	88.9 ± 2.0 B	26.17 ± 0.51 C	9.42 ± 0.18 D	<0.0001
kWh·m^{-2} ± SE	1.42 ± 0.02 A	0.89 ± 0.02 B	0.26 ± 0.01 C	0.090 ± 0.002 D	<0.0001

NaCl [g·L^{-1}] = gram per liter of NaCl in the culture medium; EC = electrical conductivity in Practical Salinity Scale (PS); Total Na^+ digestate [mg·L^{-1}] = total sodium in the digestate in milligram per liter (sodium content in the plant material plus sodium content in the inoculum); cm^3·g^{-1} = cubic centimeter per gram of volatile solids; L·m^{-2} = liter per square meter of crop production; kWh·m^{-2} = kilowatt-hour per square meter of crop production. 1 m^3 CH_4 ≈ 10 kWh. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (*p*-value ≤ 0.05) between salinity treatments are indicated by different letters (A, B, C, D, within the same row).

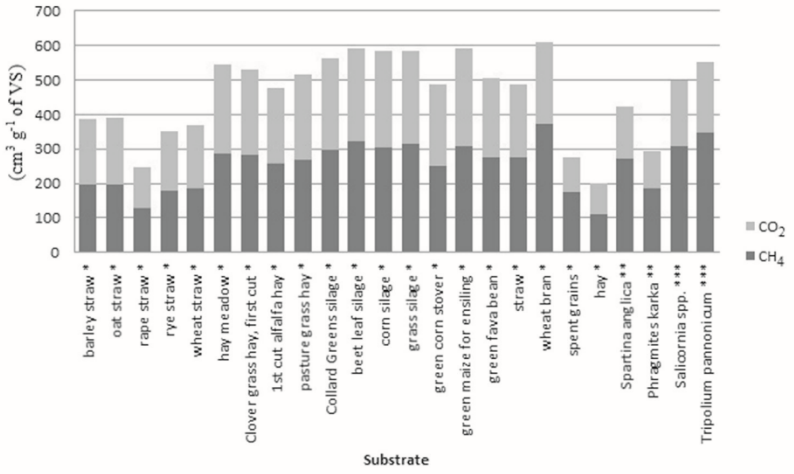

Fig. 3. Methane, carbon dioxide and total biogas from different plant species. * Data in Ref. [23]. ** Data in Ref. [24]. *** Own results: *Tripolium pannonicum* grown at 30 g L^{-1} sodium chloride.

avoided.

3.5. Sodium chloride inhibition on the anaerobic digestion process

In the conducted experiment, using substrate of E2 with different Na^+ concentrations, it is revealed that the lowest quantity of the produced methane is 301 cm^3 g^{-1} of VS with a total Na^+ concentration in the anaerobic medium of 929 mg L^{-1} (substrate + inoculum). With a Na^+ concentration of 1873 mg L^{-1}, the amount of methane produced is 306 cm^3 g^{-1} of VS, and 323 cm^3 g^{-1} of VS at 2188 mg L^{-1} of Na^+. The optimal yields of methane and biogas are obtained using the substrate of

the plants cultivated at 30 g L^{-1} NaCl, with an amount of 347 cm^3 g^{-1} of VS and 553 cm^3 g^{-1} of VS, respectively, creating a Na^+ concentration of 1988 mg L^{-1} in the total anaerobic medium. However, it was unclear whether it was due to the Na^+ concentration in the anaerobic reactors or the content of other elements and organic compounds that can cause synergistic/antagonistic effects. For example, it has being reported that at high concentrations, the antagonism phenomena due to the presence of cations and anions contained in the assayed media greatly influence the sodium effect on anaerobic sludges [26]. In another study, the combination of Na^+ and K^+ or Na^+ and Mg^{2+} increase the methane yield by about 10% compared to that produced by Na^+ alone [8]. According to these findings, sodium inhibition on the biogas yield depends on many factors such as antagonism and synergism between elements as well as the microbial adaptation to the medium. To address these questions and determine the effect of sodium on the biogas and methane yields, substrate of *T. pannonicum* cultivated under non-saline conditions was used and different amounts of NaCl were added to the anaerobic reactors (0, 440, 886, 1251 and 1746 mg L^{-1} of Na^+). According to these results, adding NaCl to the reactors inhibits the biogas and methane production (Fig. 4). By adding 440 mg L^{-1} of Na^+ (1.12 g L^{-1} NaCl), the substrate-specific biogas yield decreases from 461 cm^3 g^{-1} of VS to 411 cm^3 g^{-1} of VS representing a reduction of 10.78% with a *p*-value <0.0001. The substrate-specific methane yield decreases from 301 cm^3 g^{-1} of VS to 261 cm^3 g^{-1} of VS with a reduction of 12.98% and a *p*-value <0.0001 (Table 4). In line with these results, an early study reported sodium concentrations ranging from 3.5 g L^{-1} to 5.5 g L^{-1} to be moderately and 8.0 g L^{-1} to be strongly inhibitory to methanogenic microorganisms at mesophilic temperatures [27]. According to Feijoo et al. [26] a concentration of Na ranging from 3 g L^{-1} to 16 g L^{-1} in the absence of nutrients or other salts causes an inhibition of 50% on the methanisation, showing a higher tolerance to sodium in the sludges obtained from the digesters treating high saline wastewaters. In another study, the optimal growth conditions for mesophilic hydrogenotrophic methanogens reportedly occurred at 350 mg L^{-1} of Na^+ [28]. Based on the findings, increasing only the amount of NaCl in the anaerobic digesters inhibits the yield of biogas and methane, but these gases can be optimized with a certain amount of Na^+ in combination with other organic constituents in the substrate such as CL, HCEL, ADL, CH and/or the element content, for example K^+, Ca^{2+} and Mg^{2+}.

3.6. Microbial adaptation to salinity for biogas production

Some studies carried out have highlighted that an adaptation of the bacteria and archaea populations to the salinity is possible. For example, Feijoo et al. [26] observed a tolerance to sodium in the sludge obtained from the digesters treating high saline wastewaters and increasing the methanisation of volatile fatty acids due to the microbial adaptation to sodium. Omil et al. [29] used a sludge adapted to salinity and to ammonia contents to increase the specific methanogenic activity.

To investigate the microbial adaptation, in this study a seed sludge (inoculum) was reused that was previously adapted to different NaCl concentrations. This microbial adaptation period of the inoculum was of 67 days. *T. pannonicum* was used as substrate grown under non-saline conditions. With regard to specific biogas yield, the highest values of the factor A (inoculum adapted to different sodium concentrations) are obtained from the adapted inoculum and the lowest value from the control (no adapted inoculum). Concerning the factor B with two levels (without adding salt and adding 6.36 g L^{-1} NaCl), there is a significant difference between treatments, and the highest biogas yield is achieved where no salt is added. According to the analysis of variance, there is an interaction between factors. It means that the biogas yield depends on the adapted inoculum. The greatest amount of biogas is obtained from the salt-adapted inoculum (2.181 g L^{-1} of Na^+) without the addition of extra salt with a mean value of 438 cm^3 g^{-1} of VS. Regarding the specific methane yield, there is a highly significant difference between the levels of the factor A and B. In the factor A, the best results are achieved with the adapted inoculum, and in the factor B, the best results are obtained from the anaerobic reactors where no salt was added. The highest value of methane is 251 cm^3 g^{-1} of VS, and it is obtained using a salt-adapted inoculum (2.181 g L^{-1} of Na^+) and without the addition of extra salt. The lowest methane value is 198 cm^3 g^{-1} of VS, using the non-adapted inoculum and adding 6.36 g L^{-1} NaCl to the anaerobic reactors (Fig. 5).

The kinetics of biogas production rate from the salt adapted inoculum were also studied by using the modified Gompertz Eq. (1), which is the closest equation for biogas production in batch systems [30].

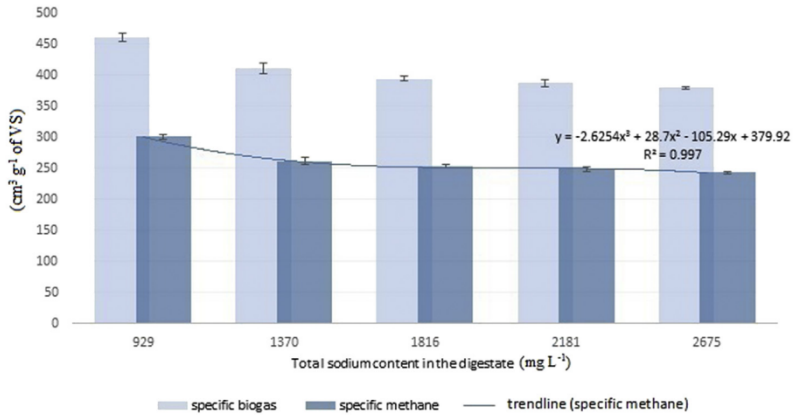

Fig. 4. Specific biogas and specific methane using *T. pannonicum* as substrate cultivated with no salt but adding NaCl to the anaerobic reactors. Columns represent mean ± SE values of 3 replicates per treatment.

Table 4
Salinity and biogas production using *T. pannonicum* as substrate cultivated under non-saline conditions but adding salt to the anaerobic reactors.

	$mg \cdot L^{-1}$ Na^+					
Added sodium	0.00	440.90	886.73	1251.94	1746.15	
Na^+ content in the substrate	492.04	492.04	492.04	492.04	492.04	
Na^+ content in the inoculum	437.51	437.51	437.51	437.51	437.51	
Total amount in the digestate	929.55	1370.45	1816.28	2181.49	2675.71	
						p-value
EC in the digestate (PS) ± SE	6.73 ± 0.03 E	7.53 ± 0.18 D	8.27 ± 0.09 C	9.27 ± 0.07 B	10.30 ± 0.06 A	<0.0001
Biogas ($cm^3 \cdot g^{-1}$) ± SE	461.1 ± 6.0 A	411.4 ± 8.6 B	394.5 ± 3.8 BC	387.6 ± 5.8 BC	379.3 ± 2.5 C	<0.0001
Biogas ($L \cdot m^{-2}$) ± SE	217.7 ± 2.8 A	194.2 ± 4.0 B	186.2 ± 1.8 BC	183.0 ± 2.7 BC	179.1 ± 1.2 C	<0.0001
Methane ($cm^3 \cdot g^{-1}$) ± SE	301.0 ± 3.9 A	261.9 ± 5.4 B	253.3 ± 2.4 BC	248.7 ± 3.7 BC	243.1 ± 1.6 C	<0.0001
Methane ($L \cdot m^{-2}$) ± SE	142.1 ± 1.8 A	123.7 ± 2.5 B	119.6 ± 1.1 BC	117.4 ± 1.7 BC	114.8 ± 0.7 C	<0.0001
$kWh \cdot m^{-2}$ ± SE	1.42 ± 0.02 A	1.24 ± 0.03 B	1.20 ± 0.01 BC	1.17 ± 0.02 BC	1.15 ± 0.01 C	<0.0001

EC = electrical conductivity in Practical Salinity Scale (PS); $cm^3 \cdot g^{-1}$ = cubic centimeter per gram of volatile solids; $L \cdot m^{-2}$ = liter per square meter of crop production; $kWh \cdot m^{-2}$ = kilowatt-hour per square meter or crop production. 1 m^3 CH_4 ≈ 10 kWh. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ≤ 0.05) between salinity treatments are indicated by different letters (A, B, C, D, E, within the same row).

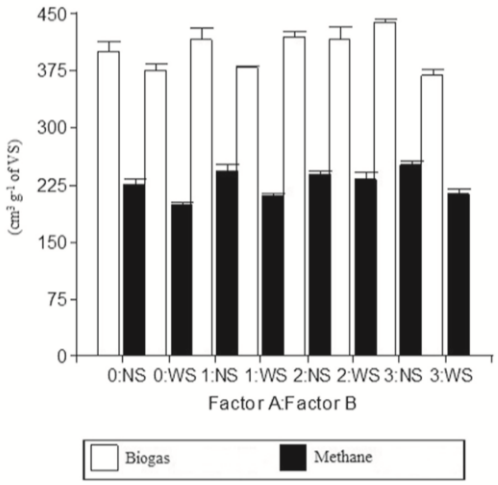

Fig. 5. Specific biogas and methane production using salt-adapted inoculum. Factor A = adapted inoculum with four concentrations of sodium (0 = control with 929 mg L^{-1}, 1 = 1370 mg L^{-1}, 2 = 1816 mg L^{-1}, 3 = 2181 mg L^{-1}). Factor B with two treatments (NS = no salt added to the anaerobic reactors, WS = 6.36 g L^{-1} of sodium chloride added to the anaerobic reactors). Values represent means in $cm^3 \cdot g^{-1}$ of VS + S.E.

$$y = A \cdot EXP\left\{ - EXP\left[\frac{U_e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, y is the cumulative of the specific biogas yield ($cm^3 \cdot g^{-1}$ of VS), A is the biogas production potential ($cm^3 \cdot g^{-1}$ of VS), U_e is the maximum biogas production rate ($cm^3 \cdot g^{-1}$ d^{-1}), λ is the lag phase period or the minimum time required to produce biogas, and t is the cumulative time for biogas production. A, λ, and U_e constants were determined using non-linear regression.

The distinct phases of the microbial growth consist of the lag phase which occurs immediately after inoculation, exponential growth phase, a deceleration phase which occurs when essential nutrients are depleted, a stationary phase during which time the net cell growth is approximately zero, and death phase. In Fig. 6, it is shown that the average specific biogas yield is very fast at the beginning of the fermentation process. This could be due to the specific growth rate of methanogenic microorganisms in the anaerobic batch. In addition, the CEL and ADL contents in the substrate are 6.7% and 18.2%, respectively, and consequently, the biodegradation is faster in comparison with other substrates with higher contents of these organic compounds, reaching 99% of the total biogas yield in the first 16 days. In terms of the typical growth curve for a batch system, after 16 days under anaerobic conditions, the biogas yield rate tends to decelerate, finally without production due to the stationary phase of microbial growth.

The average specific biogas production rate (Fig. 6) of the adapted inoculum without adding extra salt is higher than the adapted inoculum adding extra salt (6.36 g L^{-1} NaCl) but, there is not a marked difference between both kinetic biogas yield rates. This may be due to the salt-adapted microbes. These kinetic parameters U_e, A and λ using the salt-adapted inoculum without adding extra salt are 157 cm^3 g^{-1} d^{-1}, 418 cm^3 g^{-1} of VS, and 0.5 d, respectively. While using the salt adapted inoculum and adding extra salt produces the following kinetic parameters: U_e = 107 cm^3 g^{-1} d^{-1}, A = 388 cm^3 g^{-1} of VS, and λ = 0 d. In both mathematical models, the R^2 is higher than 0.993. In Fig. 6, it also can be seen that the line slope of the upper curve is sharper than the lower line. This is because the specific biogas yield rate [cm^3 g^{-1} d^{-1}] using the adapted inoculum without the addition of extra salt is higher than adding extra salt.

4. Conclusions

The salt-tolerant potential crop species *T. pannonicum* can be cultivated under saline conditions and even be watered with seawater, and subsequently its biomass can be used for biogas production. The maximum specific methane yield is obtained using the substrate of the plants grown at 22.5 g L^{-1} and 15 g L^{-1} sea-salt with a value of 313 cm^3 g^{-1} of VS and 309 cm^3 g^{-1} of VS, respectively. Using substrate of the plants cultivated with different NaCl concentrations and consequently with different Na^+ content in the plant material, it was found that the optimal production of methane and biogas is obtained using the substrate of the plants grown at 30 g L^{-1} NaCl, with an amount of 347 cm^3 g^{-1} of VS and 553 cm^3 g^{-1} of VS, respectively. The addition of NaCl to the reactors decreases the biogas and methane production. By adding 440 mg L^{-1} of Na^+ (1.12 g L^{-1} NaCl) in the reactors, the specific biogas decreases from 461 cm^3 g^{-1} of VS to 411 cm^3 g^{-1} of VS representing a reduction of 10.78%. The specific methane yield decreases from 301 cm^3 g^{-1} of VS to 262 cm^3 g^{-1} of VS with a reduction of 12.98%. An adaptation of the bacteria and archaea to the salinity to increase the methane yield is possible. The specific methane production increases from 226 cm^3 g^{-1} of VS in the control to 252 cm^3 g^{-1} of VS using the adapted inoculum with a sodium concentration of 2.181 g L^{-1}

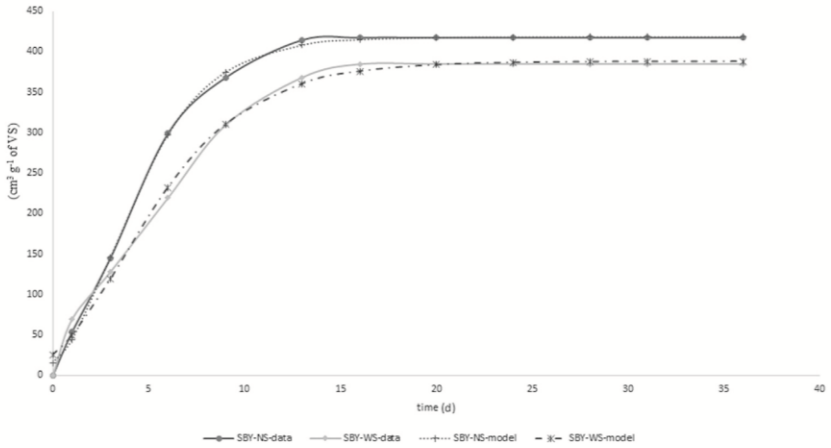

Fig. 6. Comparison of biogas production kinetics from a salt-adapted inoculum, with the addition of salt (6.36 g L^{-1} NaCl) and without the addition of salt. SBY-NS-data = specific biogas yield without the addition of salt. SBY-WS-data = specific biogas yield with the addition of salt. SBY-NS-model and SBY-WS-model = calculated yield using the kinetic model of Gompertz equation. Values of each curve represent the average of specific biogas yield between treatments.

representing an increase of 11.50%. In the anaerobic batch in which 6.36 g L^{-1} NaCl was added, the specific methane yield increases from 199 cm^3 g^{-1} of VS in the control to 232 cm^3 g^{-1} of VS in the adapted inoculum with a sodium concentration of 1.816 g L^{-1} of Na^+.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

Financial support of Ariel Turcios by the DAAD and the Universidad de San Carlos de Guatemala (Personal identification number: 91548278 funding programme: ALEGUA (57049520)) is gratefully acknowledged. We would like to thank Paul Stopp for his valuable assistance, Dr. Corinna Lorey, for her help with the gas chromatography analysis, the Institute for Sanitary Engineering and Waste Management, Leibniz University Hannover, the gardeners Yvonne Leye and Lutz Krüger, for taking care of the plants, and Hillary Cirka for correcting the English language.

Nomenclature

Acronyms

ADF om	acid detergent fiber
ADL	acid detergent lignin
CA	crude ash
CF	crude fiber
CH	total sugars
CL	crude lipids
CP	crude protein
DM	dry matter
FW	fresh weight
HCEL	hemicellulose
NDF om	neutral detergent fiber
NDL	neutral detergent lignin
PS	practical salinity (PSS-78)
VS	volatile solids

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2016.01.013.

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Chapter 4

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Potential use of the facultative halophyte *Chenopodium quinoa* Willd. as substrate for biogas production cultivated with different concentrations of sodium chloride under hydroponic conditions, Bioresource Technology, 203: 272-279. doi: 10.1016/j.biortech.2015.12.061

Bioresource Technology 203 (2016) 272–279

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Potential use of the facultative halophyte *Chenopodium quinoa* Willd. as substrate for biogas production cultivated with different concentrations of sodium chloride under hydroponic conditions

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HIGHLIGHTS

- *Chenopodium quinoa* was grown in sodium chloride with a concentration up to 30 ppt.
- The biomass composition changed due to salinity in the culture medium.
- Fermentation of *C. quinoa* led to methane yields comparable to maize.
- *C. quinoa* is a valuable crop plant suitable for seed and methane production.

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form 16 December 2015
Accepted 18 December 2015
Available online 23 December 2015

Keywords:
Anaerobic digestion
Chenopodium quinoa
Elemental analysis
Halophyte plants
Renewable energy

ABSTRACT

This project analyses the biogas potential of the halophyte *Chenopodium quinoa* Willd. In a first approach *C. quinoa* was grown with different concentrations of NaCl (0, 10 and 20 ppt NaCl) and the crop residues were used as substrate for biogas production. In a second approach, *C. quinoa* was grown with 0, 10, 20 and 30 ppt NaCl under hydroponic conditions and the fresh biomass was used as substrate. The more NaCl is in the culture medium, the higher the sodium, potassium, crude ash and hemicellulose content in the plant tissue whereas the calcium, sulfur, nitrogen and carbon content in the biomass decrease. According to this study, it is possible to produce high yields of methane using biomass of *C. quinoa*. The highest specific methane yields were obtained using the substrate from the plants cultivated at 10 and 20 ppt NaCl in both experiments.

1. Introduction

The increasing world population, with an estimated growth of 6.4 billion in 2004 to 8.1 billion in 2030, leads to an increase in the global primary energy demand by an annual average rate of 1.6% (IEA, 2006) with a projected growth of 37% by 2040 (IEA, 2014). About 83% of the overall increase in energy demand between 2004 and 2030 is met by fossil fuels (IEA, 2006). As a result, scenarios show that carbon-dioxide (CO_2) emissions, which cause global warming, will increase by 55% between 2004 and 2030 (IEA, 2006). This problem has impelled researchers in the field of green energy sources and, consequently, the development of new technological processes of energy production. Renewable resources of energy are an important issue of the worldwide battle against climate change (Oslaj et al., 2010).

Biogas production from agricultural biomass or other organic waste through anaerobic digestion is of growing importance and it is widely applied to treat various wastes and at the same time delivers renewable energy. It has been evaluated as one of the most energy-efficient and environmentally beneficial technologies for bioenergy production (Fehrenbach et al., 2008). In Germany the number of biogas plants increased from 139 in 1992 to 7900 in 2014 (Weichgrebe, 2015). This increase has led to a growing requirement for arable land for energy crops. The problem is that there is also a high demand for food crops, resulting in an overall decrease of availability of arable land for both types of crops. About 14 million hectares of land are now used for the production of biofuels, corresponding to 1% of the world's currently available arable land (IEA, 2006). Moreover, due to increasing groundwater uptake and reverse osmosis applications for drinking water, the

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http://dx.doi.org/10.1016/j.biortech.2015.12.061
0960-8524/

salt concentration in water is increasing more and more. This leads us to exploit energy crops that can be grown in land unsuitable for food crops such as coastal areas and saline soils. Halophytes are salt-tolerant plants that can grow under saline land or as biofilter to treat saline aquaculture effluents (Turcios and Papenbrock, 2014) and the biomass is an important source for bioenergy.

The aim of this research is to determine the biogas yield using the biomass from the halophyte *Chenopodium quinoa* grown under different sodium chloride concentrations [0, 10, 20 and 30 parts per thousand of sodium chloride, ppt NaCl]. The interest in this seed crop is increasing worldwide, not only because of its stress tolerance but also for its seed quality (Adolf et al., 2013) and potential for biogas production. This facultative halophytic plant can grow under salinity levels as high as those present in seawater (Adolf et al., 2013). This plant species can also be cultivated in saline land or in coastal shorelines for seed production and their crop residues (stover) are an important source for biogas production.

2. Methods

2.1. Plant biomass for biogas

Seeds of *C. quinoa* Willd. var. Titicaca were obtained from Sven-Erik Jacobsen, University of Copenhagen, Denmark, but originating in Peru, close to lake Titicaca. The agronomic handling from sowing through transplanting was carried out as described by Buhmann et al. (2015). From January to May 2015, *C. quinoa* plants were cultivated under different concentrations of NaCl, 0, 10 and 20 parts per thousand (ppt NaCl, BioChemica, AppliChem GmbH, Germany) (hereinafter called "DCR" for dry crop residues) for the purpose of seed production and the stover for biogas production. In another experiment carried out from February to April 2015 *C. quinoa* was grown under different NaCl concentrations (BioChemica), 0, 10, 20 and 30 ppt NaCl (hereinafter called "FBM" for fresh biomass) with the aim of researching the biogas yield using fresh biomass containing different salt concentrations. In the first experiment (DCR) the plants were grown for 12 weeks after transplanting in hydroponic conditions and the second one (FBM) for 5 weeks. These experiments were conducted in a greenhouse with temperatures of around 35/15 °C during day/night. 12 h of artificial light was provided (sodium vapor lamps, SON-T Agro 400, Philips). Light intensity ranged from 65 μmol m^{-2} s^{-1} to 850 μmol m^{-2} s^{-1} depending on the season, the time of the day and the weather conditions. Polypropylene containers (L400 × W300 × H175 mm) with a capacity of 16 L were used. Each container had 13.5 L solution containing 606 mg L^{-1} KNO_3, 944 mg L^{-1} $Ca(NO_3)_2 \times 4H_2O$, 230 mg L^{-1} $NH_4H_2PO_4$, 246 mg L^{-1} $MgSO_4 \times 7H_2O$, 3.73 mg L^{-1} KCl, 1.55 mg L^{-1} H_3BO_3, 0.34 mg L^{-1} $MnSO_4 \times H_2O$, 0.58 mg L^{-1} $ZnSO_4 \times 7H_2O$, 0.12 mg L^{-1} $CuSO_4 \times 5H_2O$, 0.12 mg L^{-1} $MoNa_2O_4 \times 2H_2O$, and 9.16 mg L^{-1} Fe-EDDHA (0.56 mg Fe L^{-1}). The water was aerated constantly by small compressors and one air stone in the middle of each tank (Eheim, Deizisau, Germany). The hypocotyl was fixed with soft foam in 35 mm holes. The water level was adjusted constantly in each tank with tap water to compensate the evapotranspiration and therefore the salinity was kept constant. Salinity and nutrient concentrations were monitored weekly. The cultured medium was changed two times in the DCR experiment and once in the FBM experiment. Each experimental unit consisted of eight plants per container, with three replicates per treatment.

2.2. Salinity in plant tissue

The plant tissue was incinerated using a muffle furnace (M104, Thermo Fisher Scientific Corporation, Waltham, Massachusetts, USA) pre-heated to 550 °C (ignition) during 2 h. The ashes were diluted in deionized water and the conductivity in the solution was determined in millisiemens per centimeter [mS cm^{-1}] by a pH meter (Multi 350i pH/ISE/DO/conductivity measuring instrument, Wissenschaftlich-Technische Werkstätten GmbH, Germany).

2.3. Biomass compositional analysis

The dry plant material of *C. quinoa* was ground to fine powder (MM 400, Retsch GmbH, Haan, Germany). Then 50–100 g per sample were packed into a plastic container and shipped to Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Nord-West, Oldenburg, Germany, for the determination of starch, crude lipid (CL), total sugars (CH), crude fibre (CF), Acid Detergent Fibre (ADF om), Neutral Detergent Fibre (NDF om) and Acid Detergent Lignin (ADL). CP, CL, CH, and CF were analysed according to the Commission Regulation (EC) No 152/2009, and ADF om, NDF om, and ADL according to the VDLUFA Bd. III. Crude ash (CA) was determine using a muffle furnace (M104, Thermo Fisher Scientific Corporation, Waltham, Massachusetts, USA) pre-heated to 550 °C (ignition) during 2 h. Carbon and nitrogen were determined using a C-N-S elemental analyser (Vario EL III, Elementar Analysensysteme, Hanau, Germany). Nitrogen (N) content was multiplied by the factor 6.25 to obtain crude protein (CP). The hemicellulose (HCEL) content was obtained by subtracting ADF from NDF and the cellulose (CEL) content by subtracting ADL from ADF.

2.4. Elemental analysis

For the analysis of elements in the plant material, the samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific Corporation, Waltham, Massachusetts, USA) and the procedure was carried out as described by Weese et al. (2015). For the element content in the seed sludge (inoculum), about 50 mL of homogenised material was dried at 105 °C for 24 h, then the dry material was grinded to fine powder (MM 400, Retsch GmbH, Haan, Germany). About 20 mg of powder from each sample was incinerated for 8 h in a muffle furnace (M104, Thermo Fisher Scientific Corporation). After cooling the samples to room temperature 1.5 mL of 66% nitric acid was added. After 10 min 13.5 mL of ultrapure water was added. The solution was filtered (0.45 μm pore size, Carl Roth) and stored in vials before final analysis at 4 °C. An empty vial was treated in parallel with the samples and later used as a blank. The samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific Corporation).

2.5. Biogas test

The procedure for the biogas test was conducted according to the VDI 4630 protocol (VDI, 2014). The experiment was carried out from May to July 2015 using *C. quinoa* as substrate, cultured under different concentrations of NaCl. Dry and fresh plant material was used as substrate. In the case where dry matter was used, the substrate with different salt concentrations (plants grown at 0, 10 and 20 ppt NaCl) was obtained at the end of the vegetative cycle of the plants, after harvesting the seeds. Using fresh matter, the substrate with different salt concentrations (plants grown at 0, 10, 20 and 30 ppt NaCl) was obtained during the growth phase of the plants, after 5 weeks under hydroponic conditions. Each reactor contained 200 mL of seed sludge (inoculum).

The dry matter (DM) and volatile solids (VS) contents were determined for both the substrate and the inoculum (seed sludge).

The dry matter was determined according to DIN EN (12880). The VS were determined according to DIN EN (12879).

The seed sludge as inoculum was taken on 15 May 2015 from the digester of the Sewage Treatment Plant, Hannover-Herrenhausen, Hannover, Germany. Fresh plant material (substrate) was mixed with the sludge in a ratio of 0.5 substrate/inoculum. Three replicates were used for each sample (inoculum + substrate) and three replicates as blanks (containing only inoculum). All bottles were filled with the same amount of inoculum. For each substrate the calculated amount was added to each one of the three replicate reactors.

The reactors were flushed with nitrogen for approximately 1 min to promote anaerobic conditions and after were tightly closed and incubated at 37 °C. The tests were conducted using handheld devices, taking daily pressure readings into the reactors with a manometer. Each test was terminated when the daily biogas rate was below 0.5% of the total biogas produced up to that time (36 days). After finishing the experiment, a gas sample was taken for gas chromatography analysis to determine the fraction of methane in the raw biogas samples. The biogas and methane volume was calculated in accordance to the Gay-Lussac law and converted to volume of gas under normal conditions (273.15 K and 101.325 kPa). The dissolved biogas was quantified following Henry's law.

2.6. Kinetics of the biogas production rate

The kinetics of the biogas production rate using substrate from the plants cultivated under different salt conditions were also analyzed by using the modified Gompertz equation (1), which is the closest equation for biogas production in batch systems (Yono et al., 2010).

$$y = A \cdot EXP\left\{-EXP\left[\frac{U_{max}}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where y is the cumulative of the specific biogas yield in mL_N $(g\ VS)^{-1}$, A is the biogas production potential in mL_N $(g\ VS)^{-1}$, U_{max} is the maximum biogas production rate in mL_N $(g\ VS\ day)^{-1}$, λ is the lag phase period or the minimum time required to produce biogas in days, and t is the cumulative time for biogas production in days. The A, λ, and U_{max} constants were determined using non-linear regression.

2.7. Statistical analysis

All statistical analyses were conducted using R version 3.1.1 (2014-07-10) and InfoStat software version 2014e. The Tukey multiple comparison test with $\alpha = 0.05$ was done to determine which means differ from the rest.

3. Results and discussion

3.1. Biomass yield used as substrate

In FBM, fresh biomass showed a highly significant difference between the treatments. Tukey test indicates that the plants cultivated in a salt concentration of 10 ppt NaCl produce the highest biomass, with 122.72 g $plant^{-1}$. At a salt concentration above 10 ppt NaCl the fresh weight decreases progressively, showing a negative effect on the plant growth (Fig. 1). A mean value of 8180.52 g m^{-2} is achieved at 10 ppt NaCl and a planting density of 66 plants m^{-2} (Table 1). There is a significant difference between treatments for the percentage of dry matter (DM). A comparison between means reveals that the highest value is 15.91% for the plants cultivated under non-saline conditions (control), corresponding to a dry biomass yield of about 1,041.78 g DM m^{-2}. The plants cultivated with a salt concentration of 10 ppt produce dry biomass of about 872.71 g DM m^{-2} (10.63% DM). The volatile solids decrease when the salt concentration increases in the growth medium from 83.68% in the control group to 65.83% at 30 ppt NaCl, with a yield of 871.74 g VS m^{-2} and 167.44 g VS m^{-2} respectively. As previously mentioned, the fresh biomass yield is higher at 10 ppt NaCl, but the dry biomass and volatile solids decrease under more saline conditions. However, the specific biogas yield depends on volatile solids, therefore, a higher dry biomass yield is important to evaluate the productivity of methane and biogas.

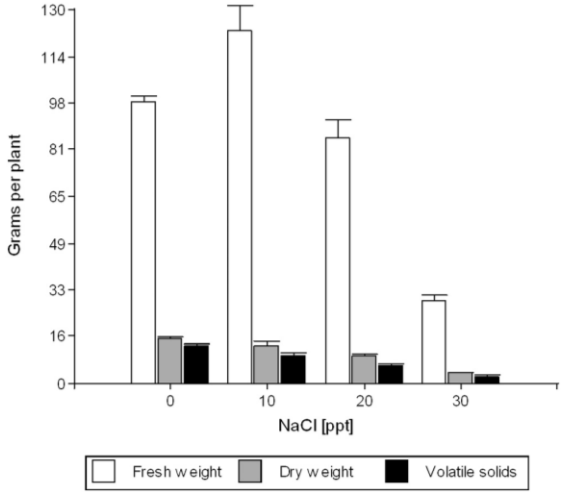

Fig. 1. Biomass yield of *C. quinoa* cultivated with different concentrations of sodium chloride (in parts per thousand ppt) for 5 weeks under hydroponic conditions. Bars represent mean and standard error of three replicates.

3.2. Seed yield

C. quinoa was able to grow, to complete its life cycle and to produce seeds in all the treatments in DCR, however, the seed yield decreased under a salinity higher than 10 ppt NaCl. The number of seeds per plant is 3466 ± 188 in the control and 3546 ± 93 at 10 ppt NaCl with no significant difference between both means but at 20 ppt NaCl the number of seeds per plant decreases significantly to 1146 ± 27 with a p-value < 0.0001. The dry seed weight in the control is 11.18 ± 2.80 g $plant^{-1}$. At 10 ppt NaCl it is 11.86 ± 0.97 g $plant^{-1}$ and with a salinity of 20 ppt NaCl it decreases significantly to 3.33 ± 1.69 g $plant^{-1}$. Additional tests were performed to determine the percentage of germination of the seeds obtained from the plants cultivated under different salt concentrations (DCR experiment). According to the obtained results, the germination percentage is higher than 95% using the seeds of the plants cultivated under non-saline conditions and at 10 ppt NaCl, while the seeds obtained from the plants cultivated at 20 ppt NaCl the germination is of 83% (data not shown).

3.3. Biomass compositional analysis

In FBM, the analysis reveals that by increased salt concentrations in the culture medium, only CA and HCEL are increased, while CP, CF, ADF, NDF, CH, CEL and starch have an inverse relationship. In DCR, the CA and CP content are higher in the plants cultivated with a salinity of 20 ppt NaCl, while the ADL content is superior

Table 1
Biomass yield of *C. quinoa* cultivated under hydroponic conditions for 5 weeks with different concentrations of NaCl.

Salinity treatment variables	0 ppt	10 ppt	20 ppt	30 ppt	*p*-value
H (cm) ± SE	73 ± 9.00^{a}	52.67 ± 3.38^{ab}	37.67 ± 1.45^{b}	14.67 ± 1.33^{c}	0.0002
SFW (g) ± SE	98.20 ± 1.5^{ab}	122.72 ± 8.71^{a}	85.15 ± 6.33^{b}	28.79 ± 1.87^{c}	<0.0001
FWSM (g m ^{2}) ± SE	6546.23 ± 99.97^{ab}	8180.52 ± 580.83^{a}	5675.88 ± 421.89^{b}	1918.92 ± 124.81^{c}	<0.0001
SDM (%) ± SE	15.91 ± 0.11^{a}	10.63 ± 0.28^{c}	11.32 ± 0.17^{c}	13.25 ± 0.13^{b}	<0.0001
DWSM (g m ^{2}) ± SE	1041.78 ± 21.76^{a}	872.71 ± 84.09^{ab}	643.27 ± 51.77^{b}	254.60 ± 18.83^{c}	<0.0001
VS (%) ± SE	83.68 ± 0.62^{a}	73.02 ± 0.15^{b}	67.09 ± 0.84^{c}	65.83 ± 0.59^{c}	<0.0001
VSSM (g m ^{2}) ± SE	871.74 ± 18.25^{a}	637.43 ± 62.4^{b}	430.74 ± 29.81^{c}	167.44 ± 11.46^{d}	<0.0001

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; H = height of plants; SFW = shoot fresh weight per plant; FWSM = fresh weight per square meter; SDM = shoot dry matter; DWSM = shoot dry weight per square meter; VS = volatile solids; VSSM = volatile solids per square meter. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ⩽ 0.05) between salinity treatments are indicated by different letters (within the same row).

Table 2
Biomass compositional analysis of *C. quinoa* cultivated under hydroponic conditions with different concentrations of NaCl.

Salinity treatment organic compounds [g (100 g DM) ^{1}]	0 ppt-FM	10 ppt-FM	20 ppt-FM	30 ppt-FM	0 ppt-DM	10 ppt-DM	20 ppt-DM
Crude ash	16.3	26.9	32.9	34.2	26.4	25.3	32.4
Crude protein	34.0	32.4	31.1	29.1	22.8	20.8	30.0
Starch	5.5	5.0	2.5	nd	2.8	3.3	2.9
Crude lipid	1.9	2.4	2.3	2.0	1.5	2.0	2.0
Total sugars	4.5	1.2	1.5	2.3	2.3	4.6	3.9
Crude fibre	12.2	9.7	8.9	7.1	15.9	16.1	15.3
ADF om	15.0	11.9	10.9	9.1	21.4	20.9	19.4
NDF om	22.4	19.9	19.3	17.9	30.7	29.8	29.5
ADL	1.6	2.0	1.6	2.1	4.8	1.8	2.0
HCEL	7.4	8	8.4	8.8	9.3	8.9	10.1
CEL	13.4	9.9	9.3	7.0	16.6	19.1	17.4

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium. Data are on dry matter basis in g (100 g DM)$^{-1}$; ADF om = Acid Detergent Fibre; NDF om = Neutral Detergent Fibre; ADL = Acid Detergent Lignin; HCEL = hemicellulose; CEL = cellulose. nd = Not determined. FM = fresh biomass as substrate from the plants cultivated for 5 weeks under hydroponic conditions (FBM); DM = crop residues (stover) as substrate from the plants cultivated for 12 weeks under hydroponic conditions (DCR).

Table 3
Electrical conductivity in the anaerobic medium, specific methane yield and productivity.

Salinity treatment variables	0 ppt-FM	10 ppt-FM	20 ppt-FM	30 ppt-FM	0 ppt-DM	10 ppt-DM	20 ppt-DM	*p*-value
EC in the plant tissue ± SE	8.80 ± 0.42^{g}	32.49 ± 0.40^{d}	43.78 ± 0.69^{c}	65.01 ± 1.65^{a}	15.05 ± 0.13^{f}	27.86 ± 0.17^{e}	52.29 ± 0.27^{b}	<0.0001
EC in the digestate ± SE	11.00 ± 0.06^{e}	13.14 ± 0.06^{d}	14.19 ± 0.19^{b}	15.40 ± 0.04^{a}	12.79 ± 0.10^{d}	13.66 ± 0.06^{c}	15.82 ± 0.01^{a}	<0.0001
Biogas [mL_N (g VS) ^{1}] ± SE	363.86 ± 7.34^{c}	467.26 ± 8.27^{a}	469.76 ± 9.85^{a}	430.08 ± 1.35^{b}	341.60 ± 3.05^{c}	453.67 ± 7.00^{ab}	458.71 ± 5.49^{ab}	<0.0001
Methane [mL_N (g VS) ^{1}] ± SE	234.97 ± 4.74^{c}	303.96 ± 5.38^{a}	304.62 ± 6.39^{a}	285.63 ± 0.89^{ab}	220.06 ± 1.96^{c}	280.10 ± 4.32^{b}	287.97 ± 3.45^{ab}	<0.0001
L_N CH_4 m ^{2} ± SE	204.98 ± 8.16^{a}	194.41 ± 22.68^{a}	131.21 ± 9.50^{b}	47.85 ± 3.41^{c}	210.57 ± 1.88^{a}	227.98 ± 3.52^{a}	119.15 ± 1.43^{b}	<0.0001
kWh m ^{2} ± SE	2.05 ± 0.08^{a}	1.94 ± 0.23^{a}	1.31 ± 0.09^{b}	0.48 ± 0.03^{c}	2.11 ± 0.02^{a}	2.28 ± 0.04^{a}	1.19 ± 0.01^{b}	<0.0001

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; EC = electrical conductivity in millisiemens per centimeter; mL_N (g VS) ^{1} = milliliters per gram of volatile solids; L_N CH_4 m ^{2} = liters of methane per square meter of crop production; kWh m ^{2} = kilowatt-hour per square meter of crop production. 1 m^3 CH_4 ≈ 10 kWh; FM = fresh biomass as substrate from the plants cultivated for 5 weeks under hydroponic conditions (FBM); DM = crop residues (stover) as substrate from the plants cultivated for 12 weeks under hydroponic conditions (DCR). Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ⩽ 0.05) between salinity treatments are indicated by different letters (within the same row).

in the control (non-saline conditions). CF, ADF, NDF and CEL are found in greater amounts in DCR than in FBM. This difference is because in DCR the plants were harvested at a more advanced growth stage, with a higher content of CF, ADF, NDF and CEL. In addition, salinity has an influence on the biomass composition (Table 2). As a result, the substrate used from the plants cultivated under different salt concentrations also influences the specific biogas and methane yield. The substrate of the plants grown under saline conditions produce more methane and biogas in comparison to the control (non-saline conditions). This is probably due to the low percentage of fibre and cellulose and a higher percentage of HCEL and CL in comparison to the control. Amon et al. (2007) found that CP, CL, CEL and HCEL have a positive influence on the methane production from maize, with a specific methane yield between 250 and 375 mL_N CH_4 $(g\ VS)^{-1}$. Starch and sugar also markedly influence the methane formation, although the role of starch for the methane yield has to be investigated in more detail (Amon et al., 2007). According to Rath et al. (2013) CL, HCEL, ADL, and CH have an influence on biogas yield, with the first two biochemical constituents being positively correlated with the biogas production and the last two showing a negative relationship. In line with this, Dandikas et al. (2014) concluded that based on the fodder analysis of different energy crops, biogas yield is significantly negatively correlated with ADL and positively correlated with HCEL. In addition, Chen et al. (2008) reported that also some other elements can enhance or inhibit the methane production such as sodium, calcium, potassium and magnesium.

3.4. Substrate salinity

Conductivity represents proportionally total ions and total ionic salinity of a sample (Zinabu et al., 2002), so the concentration of sodium chloride in the culture medium and electrical conductivity in the plant tissue (substrate) shows a direct relationship. In FBM, sodium content in the plant tissue of the plants grown under non-saline conditions is 23.96 mg $(g\ DM)^{-1}$ with electrical conductivity of 8.80 mS cm^{-1}. In the plant tissue of the plants cultivated with a concentration of 30 ppt NaCl can be observed a sodium content of 79.46 mg $(g\ DM)^{-1}$ with an electrical conductivity of 65.01 mS cm^{-1} (Table 3). This confirms that halophyte *C. quinoa*

accumulates salt in its tissues. Therefore, it is important to determine the salt concentration in the material used for anaerobic digestion, because a high salt concentration in the feedstock has influences on methanogenesis (De Baere et al., 1984), and sodium is the main inhibiting element (McCarty and McKinney, 1961), causing microbial cells to dehydrate and die due to the osmotic pressure.

3.5. Element content in the plant tissue used as substrate and in the inoculum

The concentration of NaCl in the culture medium influences the element content in the plant tissue, especially K, Na, Ca, P, Mg, S and C. K and Na content increase when the salt concentration in the culture medium increases, while Ca, S and C content decrease (Table 4). In the inoculum, the concentration of Ca, K, Na, P and S is of 1042, 902, 1456, 1081 and 247 mg L^{-1}, respectively.

The presence of various elements in the anaerobic process is essential for microbial growth. For example sodium is essential for methanogenic bacteria and archaea, presumably because it is important for the enzymatic mechanism behind the synthesis of adenosine triphosphate (ATP), or the oxidation of nicotinamide adenine dinucleotide (NADH) (Appels et al., 2008), but at high concentration may cause inhibition. Optimal growth conditions of hydrogenotrophic methanogens occur at a concentration of 350 mg Na^+ L^{-1}. Moderate inhibitory effects start at concentrations ranging from 3500 to 5500 mg L^{-1}, whereas a concentration of 8800 mg L^{-1} is strongly inhibitory to methanogenic bacteria and archaea at mesophilic temperatures (Chen et al., 2008) but adaptation can take place. In the conducted experiment the maximal concentration of Na in the anaerobic medium (substrate + inoculum), using the substrate of the plants grown at 30 ppt NaCl, is 2531 mg Na^+ L^{-1}. Although high concentrations of Na inhibits the formation of biogas (Figs. 2 and 3), interactions with other elements may occur (Chen et al., 2008). Ca, K and Mg were found below the concentration that could cause inhibitory effects on the methanation process (Jackson-Moss et al., 1989; Appels et al., 2008).

With regard to the C/N ratio, in FBM it ranges from 6.04 to 6.85 and from 7.52 to 10.03 in DCR. In the inoculum it is of 7.74 (Table 4). This *C/N* ratio is slightly below the optimum range, which is 10–30 (Amon et al., 2007). If the *C/N* ratio is below the optimal range, high total ammonia nitrogen (TAN) could be released and therefore a decrease of methanogen activity can take place (Yen and Brune, 2007). On the other hand, if the *C/N* ratio is above the optimum, carbon cannot optimally be converted into CH_4 and the CH_4 production potential cannot be fully achieved. Co-digestion of substrates could help to achieve the optimum C/N ratio and overcome this disadvantage (Amon et al., 2007).

3.6. Methane yield from C. quinoa

Using the substrate of DCR, the highest specific methane yield with a value of 288 mL_N CH_4 $(g\ VS)^{-1}$ is observed from the substrate of plants cultivated at 20 ppt NaCl. The lowest methane yield is obtained from the plants grown under non-saline conditions (control) with a value of 220 mL_N CH_4 $(g\ VS)^{-1}$. In FBM, the maximum methane production obtained using the substrate of the plants grown at 20 ppt NaCl is 305 mL_N CH_4 $(g\ VS)^{-1}$. The minimum methane production obtained using the plants cultivated under non-saline conditions has a value of 235 mL_N CH_4 $(g\ VS)^{-1}$. In both experiments, DCR and FBM, methane yield increases from the control up to 20 ppt NaCl treatment and then declines at higher salt concentrations (Table 3). In DCR and FBM, the highest methane production per area of crop production is obtained using the substrate of the plants cultivated under non-saline conditions and at 10 ppt, as well as the kilowatt-hour (kWh) per square meter of

Table 4
Element content in the biomass of *C. quinoa* used as substrate for biogas production.

Salinity treatment variable	0 ppt-FM	10 ppt-FM	20 ppt-FM	30 ppt-FM	0 ppt-DM	10 ppt-DM	20 ppt-DM	*p*-value
Ca [mg (g DM)$^{-1}$] ± SE	30.06 ± 2.09^{b}	12.92 ± 1.12^{d}	8.57 ± 0.85^{de}	3.77 ± 0.32^{f}	39.89 ± 0.27^{a}	19.12 ± 0.01^{c}	6.66 ± 0.2^{ef}	<0.0001
Cu [mg (g DM)$^{-1}$] ± SE	0.06 ± 2.10E−03^{a}	0.05 ± 0.02^{a}	0.03 ± 2.30E−03^{a}	0.03 ± 3.40E−03^{a}	0.07 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.03^{a}	0.3210
Fe [mg (g DM)$^{-1}$] ± SE	0.13 ± 0.03^{ab}	0.14 ± 0.01^{ab}	0.1 ± 3.70E−03^{b}	0.21 ± 0.04^{a}	0.11 ± 1.40E−03^{b}	0.11 ± 0.01^{b}	0.17 ± 0.01^{ab}	0.0087
K [mg (g DM)$^{-1}$] ± SE	31.86 ± 3.33^{c}	54.37 ± 8.84^{abc}	70.2 ± 1.86^{ab}	79.38 ± 2.98^{a}	55.25 ± 0.39^{abc}	28.89 ± 3.79^{c}	48.86 ± 10.86^{bc}	0.0002
Mg [mg (gDM)$^{-1}$] ± SE	1.14 ± 0.07^{d}	1.2 ± 0.05^{d}	1.58 ± 0.11^{c}	0.8 ± 0.04^{e}	2.31 ± 0.02^{a}	1.94 ± 0.01^{b}	1.38 ± 0.02^{cd}	<0.0001
Mn [mg (g DM)$^{-1}$] ± SE	0.02 ± 2.30E−03^{ab}	0.02 ± 1.40E−03 ab	0.02 ± 3.20E−03^{ab}	0.02 ± 4.00E−03^{ab}	0.01 ± 1.80E−04^{b}	0.02 ± 4.30E−04^{ab}	0.03 ± 4.50E−04^{a}	0.0072
Na [mg (g DM)$^{-1}$] ± SE	23.96 ± 3.12^{c}	56.01 ± 2.68 b	69.5 ± 4.16^{ab}	79.46 ± 2.88^{a}	19.67 ± 1.06 c	56.55 ± 0.81^{b}	71.36 ± 6.03^{ab}	<0.0001
P [mg (g DM)$^{-1}$] ± SE	5.73 ± 0.04^{de}	7.33 ± 0.01^{b}	9.51 ± 0.51^{a}	7.07 ± 0.23^{bc}	5.10 ± 0.06^{e}	6.76 ± 0.11^{bcd}	6.26 ± 0.03^{cd}	<0.0001
S [mg (g DM)$^{-1}$] ± SE	4.4 ± 0.24^{a}	3.24 ± 0.22^{b}	3.14 ± 0.4^{bc}	2.65 ± 0.29^{bc}	4.37 ± 0.05^{a}	2.32 ± 0.12^{bc}	2.11 ± 0.03^{c}	<0.0001
Zn [mg (g DM)$^{-1}$] ± SE	0.07 ± 4.70E−03de	0.09 ± 1.20E−03^{cd}	0.14 ± 0.01^{a}	0.1 ± 0.01^{c}	0.07 ± 1.30E−03^{e}	0.13 ± 2.20E−03^{ab}	0.11 ± 2.40E−03^{bc}	<0.0001
C [%]	37.28 ± 0.46^{a}	32.54 ± 0.73^{bc}	29.94 ± 0.55^{c}	29.66 ± 0.10^{c}	32.47 ± 0.06^{bc}	33.34 ± 0.05^{abc}	35.12 ± 2.33^{ab}	0.0007
N [%]	5.44 ± 0.11^{a}	5.19 ± 0.10^{a}	4.97 ± 0.26^{ab}	4.66 ± 0.18^{abc}	3.64 ± 0.01^{bc}	3.33 ± 0.02^{c}	4.81 ± 0.70^{ab}	0.0011
C/N	6.85 ± 0.05^{cd}	6.27 ± 0.04^{cd}	6.04 ± 0.21^{d}	6.37 ± 0.22^{cd}	8.92 ± 0.02^{ab}	10.03 ± 0.05^{a}	7.52 ± 0.71^{bc}	<0.0001

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; mg (g DM)$^{-1}$ = milligrams per gram of dry matter; FM = fresh biomass as substrate from the plants cultivated for 5 weeks under hydroponic conditions (FBM); DM = crop residues (stover) as substrate from the plants cultivated for 12 weeks under hydroponic conditions (DCR). Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ⩽ 0.05) between salinity treatments are indicated by different letters (within the same row).

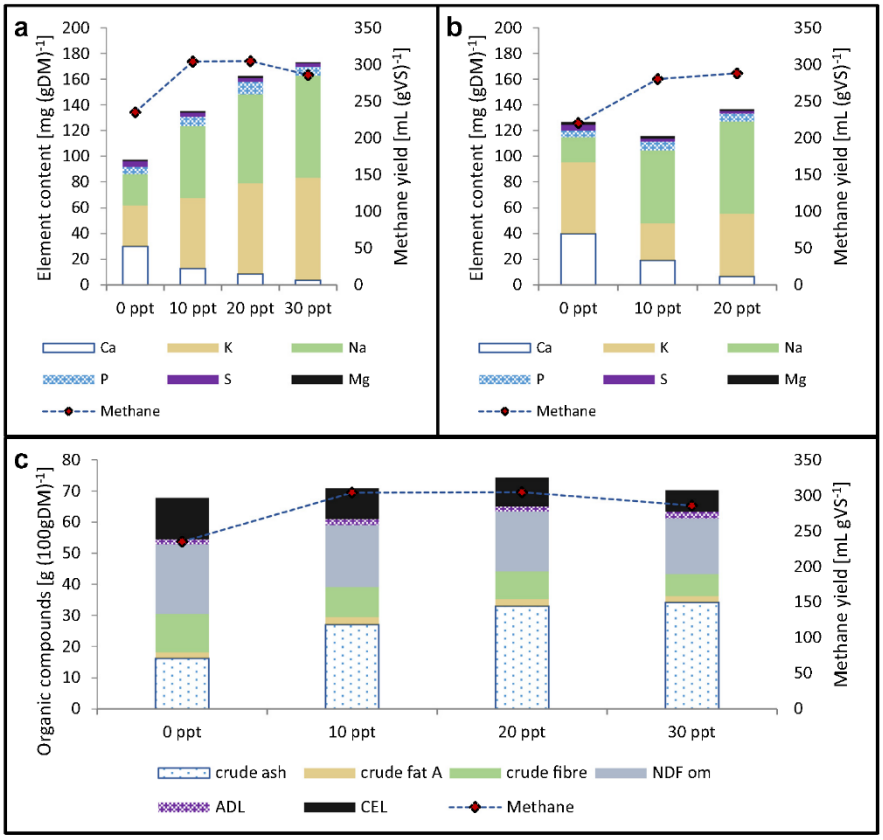

Fig. 2. Chemical composition of the substrate and specific methane yield. (a) Element content in the substrate obtained from plants cultivated for 5 weeks under hydroponic conditions (FBM); (b) element content in the crop residues (stover) of the plants cultivated for 12 weeks under hydroponic conditions (DCR); (c) organic composition of substrate obtained from plants cultivated for 5 weeks under hydroponic conditions (FBM) NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; NDF om = Neutral Detergent Fibre; ADL = Acid Detergent Lignin; CEL = cellulose. Bars represent means of three replicates.

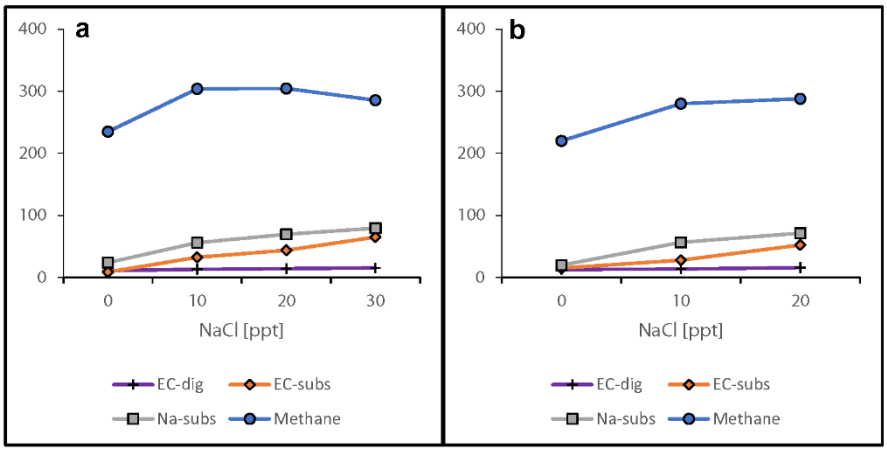

Fig. 3. Sodium content in the substrate, electrical conductivity, and specific methane yield. (a) Substrate obtained from plants cultivated for 5 weeks under hydroponic conditions (FBM); (b) crop residues (stover) of the plants cultivated for 12 weeks under hydroponic conditions (DCR). NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; EC-dig = electrical conductivity in the digestate in mS cm^{-1}; EC-subs = electrical conductivity in the substrate in mS cm^{-1}; Na-subs = sodium content in the substrate in mg $(g\ DM)^{-1}$. Lines represent means of three replicates.

Table 5
Kinetics of biogas production using the halophyte *C. quinoa* as substrate grown under hydroponic conditions for 5 weeks with different concentrations of NaCl.

Salinity treatment variables	0 ppt	10 ppt	20 ppt	30 ppt	*p*-value
A [mL_N (g VS) ^{1}] ± SE	348.63 ± 8.12^{b}	456.21 ± 7.67^{a}	461.95 ± 9.69^{a}	426.26 ± 9.02^{a}	0.0001
U_{max} [mL_N (g VS day) ^{1}] ± SE	252.04 ± 12.35^{a}	226.63 ± 2.83^{ab}	219.37 ± 5.71^{b}	215.13 ± 2.08^{b}	0.0237
λ (d) ± SE	0.58 ± 0.03^{a}	0.55 ± 0.03^{a}	0.55 ± 0.02^{a}	0.60 ± 0.03^{a}	0.5093
R^2	0.990	0.993	0.995	0.992	

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; A = biogas production potential in milliliter per gram of volatile solids; U_{max} = maximum biogas production rate in milliliter per gram of volatile solids per day; λ = lag phase period in days; R^2 = coefficient of determination of the regression model. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ⩽ 0.05) between salinity treatments are indicated by different letters (within the same row).

Table 6
Element content in the digestate (after the anaerobic process) using the halophyte *C. quinoa* as substrate cultivated under hydroponic conditions with different concentrations of NaCl.

Salinity treatment variable	0 ppt-FM	10 ppt-FM	20 ppt-FM	30 ppt-FM	0 ppt-DM	10 ppt-DM	20 ppt-DM	*p*-value
Ca [mg (g DM) ^{1}] ± SE	53.42 ± 0.44^{b}	48.13 ± 0.35^{c}	44.58 ± 0.7^{de}	42.4 ± 0.55^{e}	59.55 ± 0.51^{a}	46.44 ± 0.49^{cd}	43.95 ± 0.28^{e}	<0.0001
Co [mg (g DM) ^{1}] ± SE	0.0041 ± 0.0002^{a}	0.0039 ± 0.0002^{a}	0.0034 ± 0.0004^{a}	0.0029 ± 0.00009^{a}	0.01 ± 0.002^{a}	0.0042 ± 0.0001^{a}	0.0046 ± 0.0006^{a}	0.3812
Cu [mg (g DM) ^{1}] ± SE	0.39 ± 0.003^{ab}	0.44 ± 0.01^{a}	0.45 ± 0.02^{a}	0.43 ± 0.003^{a}	0.4 ± 0.003^{ab}	0.38 ± 0.01^{ab}	0.34 ± 0.03^{b}	0.002
Fe [mg (g DM) ^{1}] ± SE	24.69 ± 0.58^{a}	25.84 ± 0.26^{a}	24.65 ± 0.41^{a}	24.54 ± 0.03^{a}	24.59 ± 0.03^{a}	21.25 ± 0.29^{b}	21.25 ± 0.27^{b}	<0.0001
K [mg (g DM) ^{1}] ± SE	5.47 ± 0.47^{b}	11.72 ± 0.85^{b}	30.93 ± 7.58^{a}	42.68 ± 2.2^{a}	6.43 ± 2.1^{b}	5.85 ± 2.89^{b}	9.19 ± 1.25^{b}	<0.0001
Mg [mg (g DM) ^{1}] ± SE	2.69 ± 0.03^{cd}	2.86 ± 0.01^{b}	2.84 ± 0.01^{b}	2.45 ± 0.01^{e}	3.27 ± 0.01^{a}	2.78 ± 0.03^{bc}	2.66 ± 0.03^{d}	<0.0001
Mn [mg (g DM) ^{1}] ± SE	0.27 ± 0.01^{a}	0.27 ± 0.004^{ab}	0.25 ± 0.004^{cd}	0.24 ± 0.002^{d}	0.26 ± 0.001^{abc}	0.23 ± 0.002^{d}	0.25 ± 0.002^{bcd}	<0.0001
Na [mg (g DM) ^{1}] ± SE	31.55 ± 0.42^{d}	51.83 ± 1.27^{c}	50.04 ± 3.79^{c}	55.45 ± 0.32^{bc}	38.4 ± 0.68^{d}	77.72 ± 1.41^{a}	62.72 ± 2.71^{b}	<0.0001
Ni [mg (g DM) ^{1}] ± SE	0.02 ± 0.0004^{ab}	0.03 ± 0.0009^{a}	0.02 ± 0.0003^{ab}	0.02 ± 0.0002^{ab}	0.02 ± 0.0001^{b}	0.03 ± 0.001^{a}	0.02 ± 0.0002^{b}	0.0046
P [mg (g DM) ^{1}] ± SE	44.59 ± 0.36^{a}	44.6 ± 0.23^{a}	43.97 ± 0.27^{a}	44.53 ± 0.24^{a}	41.68 ± 0.71^{b}	41.87 ± 0.39^{b}	40.57 ± 0.22^{b}	<0.0001
S [mg (g DM) ^{1}] ± SE	8.52 ± 0.17^{b}	8.76 ± 0.24^{b}	8.33 ± 0.13^{b}	7.04 ± 0.25^{c}	8.86 ± 0.08^{b}	10.32 ± 0.14^{a}	8.68 ± 0.07^{b}	<0.0001
Zn [mg (g DM) ^{1}] ± SE	0.77 ± 0.02^{a}	0.8 ± 0.01^{a}	0.8 ± 0.02^{a}	0.76 ± 0.01^{a}	0.75 ± 0.0009^{ab}	0.69 ± 0.01^{bc}	0.67 ± 0.02^{c}	0.0001

NaCl (ppt) = parts per thousand of sodium chloride in the culture medium; FM = fresh biomass as substrate from the plants cultivated for 5 weeks under hydroponic conditions (FBM); DM = crop residues (stover) as substrate from the plants cultivated for 12 weeks under hydroponic conditions (DCR); mg (g DM) ^{1} = milligrams per gram of dry digestate. Values represent mean ± SE values of 3 replicates per treatment. Significant differences (p-value ⩽ 0.05) between salinity treatments are indicated by different letters (within the same row).

crop production (Table 3). The methane yield per hectare ranges from 479 to 2280 m^3_N CH_4 ha^{-1} (4790–22,800 kWh ha^{-1}). If this data is compared with other energy crops, *C. quinoa* has a high potential for methane production. For example, Amon et al. (2007) reported a maximum methane yield per hectare from late ripening maize varieties ranging from 7100 to 9000 m^3_N CH_4 ha^{-1}, and from early and medium ripening varieties 5300–8500 m^3_N CH_4 ha^{-1} when grown in favorable regions. Based on these findings, it seems that the methane yield per area is higher using maize as substrate. However, taking into account the duration of the growing cycle of the crops, in case of *C. quinoa*, the plants were cultivated under hydroponic conditions within a period of five weeks (after transplanting) with a density of planting 66 plants per square meter. As a result, the methane production per area and per year could be comparable in both crops, maize and *C. quinoa*.

3.7. Kinetics of the biogas production rate

The kinetics of the biogas production rate using substrate from the plants cultivated under different salt conditions were also analyzed by using the modified Gompertz equation (1). In all mathematical models, the coefficient of determination (R^2) is higher than 0.99, which confirms the model accuracy (Table 5).

The biogas production rate is highest in the beginning of the fermentation process. This could be due to the specific growth rate of methanogenic microorganisms in the anaerobic batch. The biodegradation rate also depends on the composition of the biomass. For example, Ay et al. (2013) found that the hydrolysis rate of the substrate increases when NDF decreases. The results of this study show that in FBM, the NDF content in the substrate gradually decreases from 22.4% in the control to 17.9% at 30 ppt-NaCl. This percentage is relatively low in comparison to other substrates of energy crops, which can be found up to 66.7% (Dandikas et al., 2014). In addition, the CEL and ADL content in the substrate ranges from 7.0% to 13.4% and from 1.6% to 2.1%, respectively. Consequently, the biodegradation rate is higher in comparison to other substrates with a larger amount of these organic compounds, reaching 99% of the total biogas yield in the first 15 days. After 15 days under anaerobic conditions, the biogas production rate in terms of the typical growth curve of a batch system tends to decelerate or stop completely due to the stationary phase of microbial growth.

There is a significant statistical difference between the treatments of FBM for the kinetic parameters U and A but not for λ. The lag phase period (λ) ranges from 0.55 to 0.60 days, the biogas production potential (A) from 348.63 mL_N $(g\ VS)^{-1}$ using the substrate from the plants cultivated under non-saline conditions (control) to 461.95 mL_N $(g\ VS)^{-1}$ using the substrate from the plants grown at 20 ppt NaCl (p-value of 0.0001). The maximum biogas production rate (U) is of 252.04 mL_N $(g\ VS\ day)^{-1}$ for the control and the lowest rate has been obtained using the substrate from the plants cultivated at 30 ppt NaCl with a value of 215.13 mL_N $(g\ VS\ day)^{-1}$ and a p-value of 0.0237 (Table 5).

3.8. Element content in the digestate

Biogas is produced during the anaerobic digestion process, which is a renewable fuel to produce heat or electricity. The residue of this process, digestate, is a valuable by-product that could be used either as foliar fertilizer, or as solid digestate and thereby improves the overall resource recovery from waste. For example, Chen et al. (2012) have outlined that 12.54 ton liquid digestate are equivalent to 118.02 kg urea, 150.00 kg potash fertilizers, and 78.68 kg phosphoric fertilizers. 5 ton solid digestate used as base fertilizers for fruit and vegetable growing are equivalent to 30.00 kg urea, 30.00 kg potash fertilizers, and 20.00 kg phosphoric

fertilizers. Although the digestate usually has a high amount of nutrients, their actual amount depends on the inoculum and feedstock used during the anaerobic process. The element content of the digestate strongly varies between anaerobic digestion assays, therefore, its content must be carefully analyzed (EBA, 2014).

According to the results of this study, potassium ranges in the digestate from 4.47 to 42.68 mg $(g\,DM)^{-1}$ using the substrate of the plants cultivated under non-saline conditions (control) and at 30 ppt NaCl, respectively. Sodium ranges from 31.55 to 77.72 mg $(g\,DM)^{-1}$ using the biomass of the control and at 10 ppt NaCl respectively. Particular attention must be given to digestate with a high content of salts and sodium, especially when used as fertilizer because of the risk of salinization and sodification of the soil. Ca, Fe and P have been found in concentrations of 42.40–59.55, 21.25–25.84 and 40.57–44.60 mg $(g\,DM)^{-1}$, respectively (Table 6). The differences between treatments is due to the different element content in the plant material used as substrate. Although most of macro-and micronutrients are conserved during the anaerobic digestion, some of them may decrease. For instance, some sulfur is converted into hydrogen sulfide during the fermentation process, therefore the final sulfur concentration in the digestate decreases. This conversion depends, among other factors, on the sulfur content in the inoculum and the substrate. Based on the results of this study, the highest concentration of hydrogen sulfide found in the biogas samples is 0.12% (1,410 ppm) using the substrate of the plants grown under non saline (control). This is because the amount of sulfur in the biomass of the plants cultivated under saline conditions is lower compared to the control, in agreement with Van Diggelen et al. (1986), possibly due to the interaction of chloride and sulfate anions in the growth medium; the uptake of sulfate is slow compared to chloride uptake. In addition, potassium analysis needs to be carefully carried out carefully because a certain amount evaporates as potassium chloride during incineration (Wang et al., 2014).

4. Conclusions

Plant material of the halophyte *C. quinoa* has a high potential for methane production. Biomass from plants cultivated with different NaCl concentrations, and consequently with a different chemical composition, has been used as substrate. The optimal specific methane yield is obtained by using the substrate of the plants cultivated at 10 and 20 ppt NaCl, with a value of 304 $mL_N\,CH_4\,(g\,VS)^{-1}$ and 305 $mL_N\,CH_4\,(g\,VS)^{-1}$, respectively. In order to obtain greater methane, biomass and seed production per unit area, it is recommended to cultivate *C. quinoa* with a salinity no greater than 10 ppt NaCl.

Conflicts of interest

None.

Acknowledgements

Financial support of Ariel Turcios by the DAAD and the Universidad de San Carlos de Guatemala is gratefully acknowledged. We would like to thank Dr. Corinna Lorey for her help with the gas chromatography analysis, Institute for Sanitary Engineering and Waste Management, Leibniz University Hannover, Annekathrin Rumlow for her help with the ICP-OES analysis, and Yvonne Leye for taking care of the plants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.12.061.

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Chapter 5

Turcios AE, Weichgrebe D, Papenbrock J, (2016): Uptake and biodegradation of the antimicrobial sulfadimidine by the species *Tripolium pannonicum* acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production, Bioresource Technology, 219: 687-693. doi: 10.1016/j.biortech.2016.08.047

Bioresource Technology 219 (2016) 687–693

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Uptake and biodegradation of the antimicrobial sulfadimidine by the species *Tripolium pannonicum* acting as biofilter and its further biodegradation by anaerobic digestion and concomitant biogas production

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HIGHLIGHTS

- *Tripolium pannonicum* was used as biofilter to uptake the antibiotic sulfadimidine (SDI).
- *T. pannonicum* was able to grow in a SDI concentration up to 10 mg·L $^{1.}$
- SDI in the culture medium was decreased by *T. pannonicum* and further during anaerobic digestion.
- Fermentation of *T. pannonicum* has led to biogas yields up to 534 mL·g ^{1} VS.
- Biomass containing SDI did not adversely affect the biogas production.

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 8 August 2016
Accepted 10 August 2016
Available online 12 August 2016

Keywords:
Anaerobic digestion
LC–MS analysis
Phytoremediation
Sulfadimidine degradation
Tripolium pannonicum

ABSTRACT

This project analyses the uptake and biodegradation of the antimicrobial sulfadimidine (SDI) from the culture medium and up to the anaerobic digestion. *Tripolium pannonicum* was grown under hydroponic conditions with different concentrations of SDI (0, 5 and 10 mg·L ^{1}) and the fresh biomass, containing different amounts of SDI taken up, was used as substrate for biogas production. SDI was analyzed by liquid chromatography coupled to positive ion electrospray mass spectrometry (ESI LC–MS). Based on the findings, *T. pannonicum* is able to uptake SDI. The more SDI is in the culture medium, the higher the SDI content in the plant tissue. According to this study, it is possible to produce high yields of biogas using biomass of *T. pannonicum* containing SDI and at the same time biodegradation of SDI is carried out. The highest specific biogas yield is obtained using shoots as substrate of the plants cultivated at 5 mg·L ^{1} SDI.

1. Introduction

The occurrence of antimicrobials in the aquatic environment has been increasing concerns. In most countries of Europe, a growing use of broad-spectrum antibiotics, such as a combination of amoxicillin and clavulanic acid, new macrolides, quinolones, cephalosporins, penicillins, tetracyclines (TCs) and sulfonamides (SAs), has been observed (Goossens et al., 2005). Tetracyclines and SAs are used mainly as veterinary medicines against bacteria and as growth promoters. These antibiotics are poorly absorbed in the gut and consequently a substantial amount is excreted in urine and feces. As much as 90% of some SAs may be excreted as the parent compound (Kim et al., 2011). These residues are entering the environment via effluent discharges, reaching other water bodies and, as a result, their presence is increasingly being recognized as the main cause of emerging antimicrobial-resistant bacteria (Lindsey et al., 2001). Some antibiotics have been found in the source waters of drinking water treatment plants, including sulfamethoxazole (3.0–3.4 ng·L^{-1}), macrolides (1.4–4.9 ng·L^{-1}), and quinolones (1.2–4.0 ng·L^{-1}) (Ye and Weinberg, 2007). Lindsey et al. (2001) reported TCs and SAs concentrations ranging from 0.07 to >15 μg·L^{-1} in groundwater and surface water, while in

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http://dx.doi.org/10.1016/j.biortech.2016.08.047
0960-8524/

different liquid manure samples sulfadimidine (SDI) has been found in the range of 1–2 mg·kg^{-1} (Christian et al., 2003), and in some German biogas plants the presence of several antibiotics, including SDI, with concentrations up to 9 mg·kg^{-1} have been reported (Spielmeyer et al., 2014).

Several studies have proven that some pharmaceuticals are not eliminated completely during conventional wastewater treatment and being discharged into receiving waters (Gros et al., 2012; Ternes et al., 2004). Therefore, it is important to use efficient and low-cost technologies which allow the removal of these compounds. Plants acting as biofilter to uptake organic pollutants have a potential use, being a cost-effective method for treating wastewater (Turcios and Papenbrock, 2014). Because wastewater contains dissolved salts with concentrations ranging from 10 g·L^{-1} up to 71 g·L^{-1} in some effluents treated anaerobically (Lefebvre and Moletta, 2006), selected plants need to be tolerant to salinity in order to have a resilient and effective biofiltration system (Calheiros et al., 2012). *Tripolium pannonicum* is a halophyte, which can withstand salty conditions up to 45 g·L^{-1} NaCl (Turcios et al., 2016a). Additionally, this plant species performs very well under hydroponic conditions where saline water containing low concentrations of nitrates and phosphates (10 mg·L^{-1} N and 0.3 mg·L^{-1} P, respectively) do not limit biomass production and biofilter capacity (Buhmann et al., 2015), and also withstands repetitive harvesting (Ventura et al., 2013). In this context, the salt-tolerant plant species *T. pannonicum* was tested in order to decrease the content of the antimicrobial SDI under hydroponic conditions. Moreover, this plant species is able to uptake other chemicals pollutants such as nitrates and phosphates (Buhmann and Papenbrock, 2013; Buhmann et al., 2015), thus the potential eutrophication of receiving water bodies can be avoided. The antimicrobial SDI was chosen due to its high use in livestock, being very commonly found in the environment and on the other hand, because it is poorly adsorbed, being more precise to follow the uptake and biodegradation process. Another concern could be the use of the plant biomass after acting as biofilter. For this reason, the further use of this plant material for biogas production is proposed, being a renewable energy. By year 2020, 20% of the energy demand within the European Union should be generated by renewable energy systems such as solar, wind, or biomass (Spielmeyer et al., 2014). In addition, during the anaerobic digestion biodegradation of antibiotics may take place. Only limited information is available about the elimination of antibiotics within the fermentation process, and so far it is not completely clear if degradation takes place.

In this context, the main objective of this study is to evaluate the uptake and biodegradation of SDI by the plant species *T. pannonicum*, its distribution in the plant, its further biodegradation under anaerobic conditions as well as to evaluate the effect of SDI on biogas production.

2. Materials and methods

2.1. Plant cultivation

The seeds of *Tripolium pannonicum* (Jacq.) Dobrocz were collected at the North Sea, Germany (53°29′13″N; 8°03′16″E). The agronomic handling from sowing through transplanting was carried out as described by Buhmann et al. (2015). From November 2015 to January 2016, *T. pannonicum* plants were grown under different SDI concentrations (0, 5 and 10 mg·L^{-1}). All plants were grown for five weeks after transplanting in hydroponic conditions. These experiments were conducted in a greenhouse at the Institute of Botany, Leibniz University Hannover, Germany (52°23′42″N; 9°42′13″E), with temperatures between 14 °C (minimum temperature during the night) and 35 °C (maximum temperature during the day). Plants were exposed to 12 h of artificial light (sodium vapor lamps, SONT Agro 400, Philips). Light intensity ranged from 65 μmol·m^{-2}·s^{-1} to 850 μmol·m^{-2}·s^{-1} depending on the time of the year, the time of the day and the weather conditions. Polypropylene containers (400 mm × 300 mm × 175 mm) with a capacity of 16 L were used. Each container had 13 L solution containing 606 mg·L^{-1} KNO_3, 944 mg·L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 230 mg·L^{-1} $NH_4H_2PO_4$, 246 mg·L^{-1} $MgSO_4{\cdot}7H_2O$, 3.73 mg·L^{-1} KCl, 1.55 mg·L^{-1} H_3BO_3, 0.34 mg·L^{-1} $MnSO_4{\cdot}H_2O$, 0.58 mg·L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.12 mg·L^{-1} $CuSO_4{\cdot}5H_2O$, 0.12 mg·L^{-1} $MoNa_2O_4{\cdot}2H_2O$, and 9.16 mg·L^{-1} Fe-EDDHA (0.56 mg·L^{-1} Fe). The water was constantly aerated by small compressors and one air stone in the middle of each tank (Eheim, Deizisau, Germany). The hypocotyl was fixed with soft foam in 35 mm holes. The water level was adjusted constantly in each tank with tap water to compensate for evapotranspiration. Each experimental unit consisted of eight plants per container, with three technical replicates per treatment. Three extra containers under the same conditions (0, 5 and 10 mg·L^{-1} SDI) but without plants were used, with the purpose of monitoring the SDI degradation due to environmental conditions. After harvesting, different parts of the plants (young leaves, old leaves, stem, shoots and roots) were washed with deionized water, water was removed using blotting paper, and immediately frozen in liquid nitrogen. Plant samples were stored at −80 °C before extraction.

2.2. Plant sample preparation

After using twenty-one methods in order to optimize the SDI extraction (Table A.2), the following procedure was applied. Plant samples were ground in liquid nitrogen to a fine powder. About 100 mg of fresh and crushed plant material was weighted in 2 mL reaction tubes. Then for SDI extraction an acid hydrolysis was performed. Extraction buffer was added (1000 μL, 1% formic acid in MeOH v/v). The samples were shaken at 1000 min^{-1} at room temperature for 4 h (Eppendorf® Thermomixer Compact, Hamburg, Germany). Then 1000 μL of hydrolysis buffer was added (10% HCl in MeOH v/v) and heated at 85 °C for 40 min. After cooling, the samples were centrifuged for 5 min at 10,000 min^{-1} (9279*g*). For samples from the aerial parts of the plants, 500 μL of the supernatant was diluted in 500 μL 80% MeOH. For samples from roots, a final dilution of 1:200 was done (in 80% MeOH). Internal standard (simeton) was added to the samples to reach a final concentration of 100 ng·mL^{-1}.

Control samples were used during the extraction process because a temperature of 85 °C could cause some losses of the antibiotic substance. The controls contained the same amount of plant material as the samples, but plants grown in the absence of antibiotic were used. To these controls, a certain amount of antibiotic was added and handled in the same manner as the rest of samples during the extraction process.

2.3. Methanogenic activity test

The procedure for the biogas test was conducted according to the VDI 4630 protocol. The experiment was carried out from February 2016 to March 2016. Fresh plant material from *T. pannonicum* as substrate was used, cultivated under different SDI concentrations (0, 5 and 10 mg·L^{-1}). In parallel, a second experiment was conducted using *T. pannonicum* as substrate, but cultivated without SDI. In this experiment, different amounts of SDI were added to the anaerobic reactors (0, 0.1 and 0.5 mg·L^{-1}). Glass bottles, with a volume of 1000 mL were used as the testing batch reactors. Each reactor contained 200 mL of media (sludge pellets, deionized water and substrate). The dry matter (DM) and volatile solid (VS) contents were determined for both the substrate and the inoculum (sludge pellets).

The dry matter was determined according to DIN EN 12880. The VS contents were determined according to DIN EN 12879.

The pellets used as inoculum were taken from the digester of the wastewater treatment plant, riha WeserGold Getränkegruppe, Behrenstraße 44-6, 31737 Rinteln, Germany. Fresh plant material (substrate) was mixed with the inoculum in a ratio of 0.5 VS substrate/VS inoculum. Three replicates were used for each sample (containing inoculum, substrate and deionized water) and three replicates as blanks (containing only inoculum and deionized water). All bottles were filled with the same amount of inoculum. For each substrate, the calculated amount was added to each one of the three replicate reactors.

The reactors were flushed with nitrogen for approximately 1 min to promote anaerobic conditions and after were tightly closed and incubated at 37 °C. The tests were conducted using handheld devices, taking daily pressure readings into the reactors with a manometer. Each test was terminated when the daily biogas rate was below 0.5% of the total biogas produced up to that time (30 d). After finishing the experiment, a gas sample was taken for gas chromatography analysis to determine the fraction of methane in the raw biogas samples. The biogas and methane volumes were calculated in accordance to the Gay-Lussac law and converted to volume of gas under normal conditions (273.15 K and 101.325 kPa). The dissolved biogas into the liquid phase was quantified and calculated following Henry's law, respectively.

2.4. Digestate sample preparation

After 30 d under anaerobic conditions, samples were homogenized and collected in 15 mL polypropylene centrifuge tubes (Sarstedt AG & Co, Nümbrecht, Germany). For extraction, 1 mL sample was transfer into a 2 mL reaction tube and managed in the same manner as the plant tissues. 500 µL extraction buffer was added (2% formic acid in MeOH v/v). The samples were shaken at 1000 min^{-1} at room temperature for 4 h (Eppendorf® Thermomixer Compact). Then 500 µL hydrolysis buffer was added (20% HCl in MeOH v/v), heated to 85 °C for 40 min. After cooling, the samples were centrifuged for 5 min at 10,000 min^{-1} (9279g). For the digestate containing the aerial part of the plant as substrate, 500 µl of the supernatant was diluted in 500 µl 80% MeOH. Digestate containing roots as substrate, a final dilution of 1:200 was done (in 80% MeOH). Internal standard (simeton) was added to the samples to have a final concentration of 100 $ng{\cdot}mL^{-1}$. Control samples were used in the same way as described above (Section 2.2). In order to study the matrix effect, certain concentrations of SDI were added to the anaerobic medium and then the concentration of SDI was quantified.

2.5. Water sampling procedure

During the experiment, samples were collected from the culture medium in which plants were grown with the first sampling at the day of planting and the last sampling at the day of harvesting. In order to study the effect of the environmental conditions on the antibiotic, water samples were also taken at the beginning and at the end of the experiment from other three containers under the same conditions (0, 5 and 10 $mg{\cdot}L^{-1}$ SDI) but without plants. Source water samples were filtered through 0.22 µm pore size (Carl Roth, Karlsruhe, Germany) before storage at −70 °C. For LC–MS analysis, the samples were diluted (1:100) in 80% methanol (MeOH), LC–MS grade. Also the effect of the different matrixes was studied. For this purpose, certain concentrations of SDI were added to the culture medium where the plants were grown and then samples were analyzed by LC–MS.

2.6. LC/MS conditions, quantitation and linearity

Samples were analyzed by a high-performance liquid chromatography (HPLC) system (Shimadzu, Canby, USA), equipped with two solvent delivery units (LC-20AD_{XR}), an autosampler (SIL-20AC_{XR}), a photodiode array detector (SPD-M20A), and a column oven (CTO-20AC). Analytes were separated using a Vertex Plus column (250 × 4 mm, 5 µm pore size, packing material ProntoSIL 120-5 C18-H) with a corresponding precolumn (Knauer, Berlin, Germany). The analytes were separated and identified within 20 min, therefore a total run time of 30 min per sample was allowed. A binary gradient with a flow rate of 0.80 $mL{\cdot}min^{-1}$ was used. Mobile phase A contained 2 mM ammonium acetate in ultrapure water. Mobile phase B contained 2 mM ammonium acetate in MeOH. For separation, the initial mobile phase containing 10% B was increased linearly to 90% B in 25 min, which was held for 2 min before decreasing linearly to 10% B in 1 min and holding for an additional 2 min to allow reequilibration of the column. The eluted compounds were analyzed by UV/Vis spectra from 190 to 800 nm using a deuterium lamp. The HPLC system was coupled to an AB Sciex Triple TOF 4600 mass spectrometer (AB Sciex Deutschland GmbH, Darmstadt, Germany). Positive electrospray ionization (ESI) was used at a nebulizer temperature of 600 °C and an ion spray voltage floating of 4500 V. Mass spectra in the range of 120–930 Da were measured in the TOF range, in addition MS/MS spectra from 50 to 800 Da at a collision energy of 30 V were recorded.

Quantitation was based on a detector response defined as the area ratio of the base peak ion to the base peak ion of the internal standard (MultiQuant™ Software, AB Sciex). The calibration curve was constructed by plotting the area ratio of the ion response of the external standard (SDI vetranal™) and the internal standard (simeton) against the ratio of the spiked concentrations. The internal standard simeton was used to compensate for the variation of the volumes of the final extracts and to check the instrumental performance. The limits of detection (LOD) and quantification (LOQ) were determined according to the DIN EN 32645, 2008. For identification and quantitation of non-target metabolites, MasterView™ Software (AB Sciex) was used. Linearity was tested with concentrations from 5 to 200 $ng{\cdot}mL^{-1}$ SDI containing a concentration of 100 $ng{\cdot}mL^{-1}$ of simeton as internal standard (Fig. A.1).

2.7. Statistical analysis

All statistical analyses were conducted using R, version 3.1.1 (R Core Team, 2014) and InfoStat software, version 2016e (Rienzo et al., 2016). The Tukey multiple comparison test with $\alpha = 0.05$ was done to determine which means differ from the rest.

2.8. Chemicals and reagents

Both analytical standards, simeton Pestanal® (99.8%) and sulfadimidine Vetranal™ (99.7%), were of high purity grade (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Sulfadimidine (⩾99%) used in the culture and in the anaerobic medium was purchased from Sigma-Aldrich Chemie GmbH. Methanol LC–MS Chromasolv® was purchased from Fluka (Sigma-Aldrich Chemie GmbH). Stock standards were prepared on a weight basis in methanol (at a concentration of 1 $g{\cdot}L^{-1}$) and stored at −20 °C for no longer than 2 months.

3. Results and discussion

3.1. Linearity, quantitation, and precision

Standard calibration curve shows linear response of two orders of magnitude (correlation coefficient >0.99). The limits of detection

(LOD) and quantification (LOQ) are 0.278 and 0.995 ng·mL^{-1}, respectively. The lowest quantified value in the samples was 2.6 ng·mL^{-1}, which is above the LOQ. Inter-day precision was determined by repeated injection (n = 3). The maximum percent Coefficient of Variation (% CV) is 11.78% at 10 ng·mL^{-1} SDI (Table A.1). According to EU Commission Decision 2002/657/EC6, this value falls within acceptable limits (20% at ⩾10 μg·kg^{-1} to 100 μg·kg^{-1}).

3.2. Biomass yield of T. pannonicum cultivated under different concentrations of SDI

The presence of SDI in the hydroponic growth medium adversely affects the plant growth. The higher the SDI concentration in the culture medium, the lower the fresh and dry biomass of shoots and roots, with significant differences between treatments (Table 1). Tukey test indicates that the plants cultivated without SDI produce the highest shoot biomass, with 44.05 ± 0.89 g per plant (n = 3), and the lowest value is of 20.45 ± 3.17 g per plant at 10 mg·L^{-1} SDI. On the contrary, the percentage of dry matter increases at higher concentrations of antibiotic; therefore, the dry matter production shows slightly significant differences between treatments (Table 1). The shoot to root fresh biomass ratio is significantly higher at 10 mg·L^{-1} than in the control, with values of 5.49 ± 0.56 and 2.83 ± 0.20, respectively, indicating that the roots are more sensitive than the shoots at higher concentrations. Volatile solid yields (organic total matter) do not show any significant difference, but tend to decrease when the SDI concentration increases in the growth medium. Plants cultivated in the absence of SDI produced 174.54 ± 7.97 g·m^{-2} VS, while those cultivated at 5 mg·L^{-1} SDI produced 148.98 ± 16.12 g·m^{-2} VS, and 116.09 ± 13.56 g·m^{-2} VS at 10 mg·L^{-1} SDI. As previously mentioned, the fresh biomass yield is higher in the control group, but the percentage dry biomass and percentage volatile solids increase in the presence of SDI. In addition, the biogas yield depends on volatile solids, therefore, a higher dry biomass yield is important to evaluate the productivity of methane and biogas.

The reduction of fresh biomass yield could be as part of stress-response in plants caused by the antibiotic. Certain contaminants in the culture medium can cause plant stress and induce defense mechanisms to overcome the negative effects. Some plants species are tolerant to antimicrobials either by selective uptake mechanisms, dissipation of the antibiotic in the rhizosphere, or by a release of stress response hormones (Mathews and Reinhold, 2013). This tolerance could also depend on the antimicrobial itself and its concentration. SDI interferes with folic acid synthesis via inhibition of para-aminobenzoic acid metabolism and consequently decreases the amount of folate in the plant (Zhang et al., 2012). This can lead to a decrease in fresh biomass yield. The relative increase in dry biomass and volatile solids in the presence of SDI could be due to developed biochemical mechanisms to deal with the stress such as alteration in the cell wall, cytoskeleton and membrane structure, whereas the water content in the cells decreased.

3.3. Uptake of SDI by the T. pannonicum plants

Based on the results, *T. pannonicum* is able to uptake the antimicrobial SDI. The amount of antibiotic found in plant material depends on the plant part from which it is extracted, indicating that this antibiotic has different distribution patterns in the plant. The highest amount is found in the roots with values of 213.37 ± 5.14 μg·g^{-1} FM and 313.57 ± 7.72 μg·g^{-1} FM at 5 and 10 mg·L^{-1} SDI, respectively. In the aerial parts, the highest quantity is found in the stem, changing significantly from 5.18 ± 1.76 μg·g^{-1} FM at 5 mg·L^{-1} SDI to 12.04 ± 2.54 μg·g^{-1} FM at 10 mg·L^{-1} SDI. The lowest amount is found in the young leaves with a value of 0.22 ± 0.05 μg·g^{-1} FM at 5 mg·L^{-1} SDI and 0.87 ± 0.28 μg·g^{-1} FM at 10 mg·L^{-1} SDI (Fig. 1). In the control group (plants cultivated without antibiotic) no SDI is found. In addition, these findings suggest that the SDI content in the plant material not only depends on the plant part but also on the concentration in the culture medium: the higher SDI the concentration in the culture medium, the greater the amount found in the plant material, which could indicate that this antibiotic is absorbed and transported in a passive way, without spending extra energy. The high amount found in the stem could be due to the low octanol/water partition coefficient (K_{ow}) of SDI (log K_{ow} = 0.14), which means that SDI is highly soluble in the aqueous phase. Therefore it would be expected to be easily transported via the xylem vessels. The lower concentration in the leaves could be due to a low permeability and limited symplastic transport via the endodermis (Burken, 2003). It could also be either the bound SDI being non-extractable from the plant tissues and thus, elude analysis or due to the biodegradation of this organic compound by the plants.

According to Mathews and Reinhold (2013), there are three phases of plant metabolism of organic contaminants: Phase I, transformation of organic contaminants; Phase II, conjugation of parent contaminants and Phase I metabolites; and Phase III,

Table 1
Biomass yield of *T. pannonicum* cultivated under different concentrations of sulfadimidine (SDI).

SDI concentration	mg L^{-1}			*p*-value
	0	5	10	
Variable				
SFM (g/plant) ± SE	44.05 ± 0.89 a	29.55 ± 4.79 ab	20.45 ± 3.17 b	0.0072
RFM (g/plant) ± SE	15.67 ± 0.74 a	6.03 ± 1.15 b	3.73 ± 0.52 b	0.0001
SFM/RFM ratio ± SE	2.83 ± 0.20 b	4.95 ± 0.28 a	5.49 ± 0.56 a	0.0058
SDM (%) ± SE	7.51 ± 0.34 b	9.4 ± 0.87 ab	10.65 ± 0.44 a	0.0267
RDM (%) ± SE	5.87 ± 0.13 c	13.04 ± 0.56 b	15.8 ± 0.65 a	<0.0001
SVS (%) ± SE	79.24 ± 1.91 a	82.47 ± 3.69 a	81.28 ± 3.58 a	0.7750
RVS (%) ± SE	85.67 ± 1.91 a	89.13 ± 1.13 a	90.64 ± 1.39 a	0.1365
SFM (g·m^{-2}) ± SE	2936.29 ± 59.05 a	1969.64 ± 319.59 ab	1362.92 ± 211.49 b	0.0072
RFM (g·m^{-2}) ± SE	1044.4 ± 49.57 a	402.24 ± 76.51 b	248.59 ± 34.85 b	0.0001
SDM (g·m^{-2}) ± SE	220.09 ± 5.48 a	179.68 ± 11.69 ab	143.25 ± 17.19 b	0.0135
RDM (g·m^{-2}) ± SE	61.34 ± 3.68 a	51.78 ± 7.98 a	38.93 ± 4.23 a	0.0791
SVS (g·m^{-2}) ± SE	174.54 ± 7.97 a	148.98 ± 16.12 ab	116.09 ± 13.56 b	0.0512
RVS (g·m^{-2}) ± SE	52.58 ± 3.59 a	46.33 ± 7.77 a	35.17 ± 3.25 a	0.1396

SDI, sulfadimidine in milligram per liter; SFM, shoot fresh matter; RFM, root fresh matter; SDM, shoot dry matter; RDM, root dry matter; SVS, shoot volatile solids; RVS, root volatile solids. Values represent mean ± SE values of three technical replicates per treatment. Significant differences (*p*-value ⩽ 0.05) between treatments are indicated by different letters.

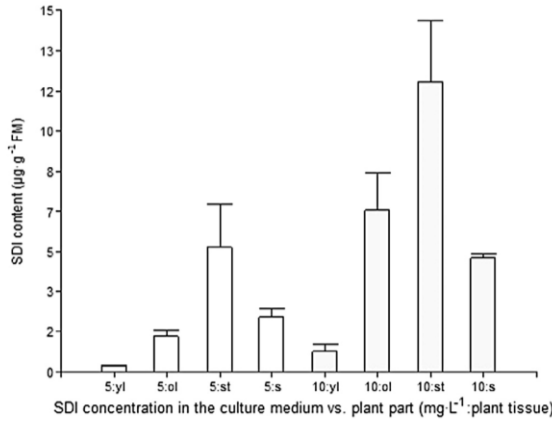

Fig. 1. Sulfadimidine content in the plant tissues. SDI = Sulfadimidine; 5 = 5 mg·L^{-1} SDI in the growth media; 10 = 10 mg·L^{-1} SDI in the growth media; yl = young leaves; ol = old leaves; st = stem; s = shoots. Columns represent mean ± SE values of 3 technical replicates per treatment.

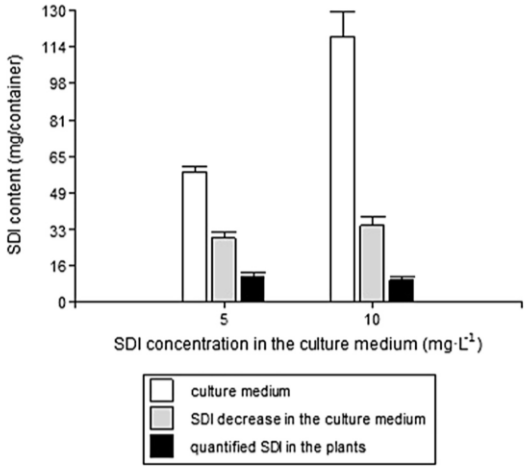

Fig. 2. Sulfadimidine balance in the growth media. SDI = Sulfadimidine. Columns represent mean ± SE values of 3 technical replicates per treatment.

sequestration or compartmentalization of Phase II metabolites. Burken (2003) reported that hydroxylation is the most prevalent metabolic process for organic compounds in plants. N-glycosidation is a likely mechanism of plant metabolism of SAs, with some potential metabolites such as N^4-acetyl-sulfonamides and N^4-hydroxy-sulfonamides with their likely further conjugation through *O*-glycosyltransferases or glutathione-*S*-transferases, although conjugation of SAs with glutathione directly also could occur (Mathews and Reinhold, 2013).

To complement this analysis, a SDI mass balance was carried out. In the plant growth media with an initial SDI concentration of 65 mg per container (5 mg·L^{-1}), a SDI decrease of 28.25 ± 2.81 mg per container (2.17 ± 0.21 mg·L^{-1}) is quantified, while in the total plant material 10.88 ± 2.15 mg is found, representing about 39% of the decreased total amount in the growth media. In the plant growth media with an initial concentration of 130 mg per container (10 mg·L^{-1} SDI), a reduction of 34.15 ± 3.76 mg per container (2.63 ± 0.29 mg·L^{-1}) is observed, of which 10.06 ± 1.13 mg is found in the plant tissues (Fig. 2). Although the concentration of SDI in the plants cultivated at 10 mg·L^{-1} SDI is higher than those cultivated at 5 mg·L^{-1} SDI, the total amount found in both conditions is almost the same due to the lower biomass productivity at 10 mg·L^{-1} SDI. In the containers where no plants were cultivated, no significant reduction of SDI is quantified, consequently this SDI decrease in the growth media is due to the uptake by the plants, though some SDI modification or degradation may occur in the rhizosphere as a result of the presence of microorganisms or root exudates (Lin et al., 2010). This could be confirmed by studying the microbial activity in the rhizosphere and by measuring the reactive oxygen species (ROS), as Gujarathi and Linden (2005) suggest that ROS increase in the culture medium when plants are under stress.

3.4. Sulfadimidine degradation during the anaerobic digestion

A significant SDI decrease during the anaerobic digestion is quantified. After anaerobic digestion, using plant material (shoots) as substrate with a SDI content of 2.32 ± 0.34 µg·g^{-1} FM, a final SDI concentration of 0.97 ± 0.09 µg·g^{-1} FM is found. Shoots with a SDI content of 4.72 ± 0.16 µg·g^{-1} FM, an amount of 1.98 ± 0.20 µg·g^{-1} FM after anaerobic digestion is found, which represents a decrease of about 58%. Roots were used for biogas production as well, with

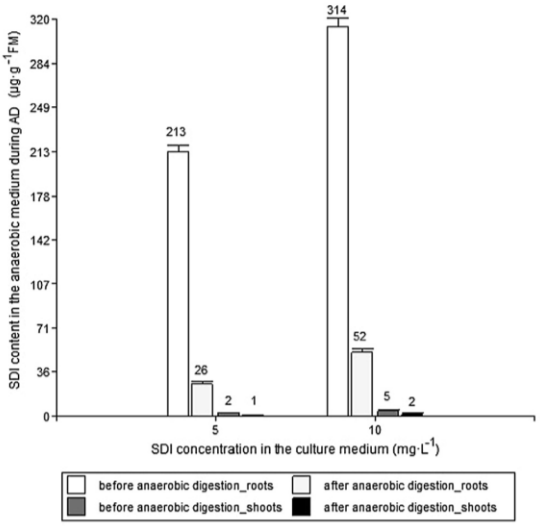

Fig. 3. Sulfadimidine content in the plant material before and after anaerobic digestion. SDI = Sulfadimidine. AD = Anaerobic digestion. Columns represent mean ± SE values of 3 technical replicates per treatment.

different contents of SDI. Roots with an initial content of 213.37 ± 5.14 µg·g^{-1} FM and 313.57 ± 7.72 µg·g^{-1} FM, a final content of 26.39 ± 1.42 µg·g^{-1} FM and 51.64 ± 2.89 µg·g^{-1} FM is found respectively. No SDI is found in the anaerobic reactors where plant material without antimicrobial as substrate is used. In addition, a significant SDI decrease is quantified in the anaerobic reactors where SDI is added. In anaerobic reactors, in which 20 µg SDI is added (100 ng·mL^{-1}), the final quantified concentration is 15.35 ± 0.53 ng·mL^{-1}, and in those where 100 µg SDI is added (500 ng·mL^{-1}) yields a final concentration of 124.85 ± 3.21 ng·mL^{-1}. Based on these findings, a significant amount is degraded during anaerobic digestion (Fig. 3). Although, due to some particles in the anaerobic media also adsorption processes may take place. However, in the spiked samples no matrix effect is observed. In addition, during the whole process, the extracts

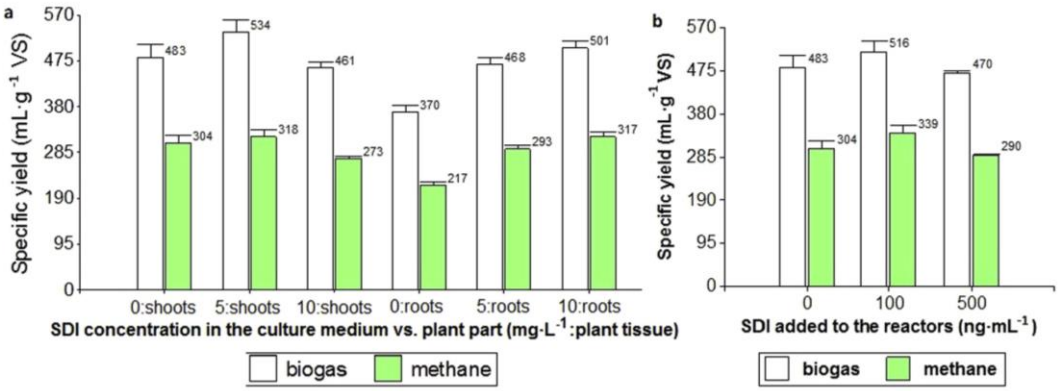

Fig. 4. Biogas and methane production a) Biogas and methane production using roots and shoots as substrate of the plant species *T. pannonicum* cultivated with different concentrations of SDI (0, 5 and 10 mg·L^{-1}). b) Biogas and methane production using shoots as substrate of the plant species *T. pannonicum* cultivated in the absence of SDI and adding different concentrations of SDI to the anaerobic reactors (0, 100 and 500 ng·mL^{-1}). Columns represent mean ± SE values of 3 technical replicates per treatment.

were checked for the most common SDI metabolites (N^4-acetyl-SDI, N^4-hydroxy-SDI, desamino-SDI, N^4-glucose conjugate, among others). Although no significant peaks for these known metabolites are observed, also the formation of other unknown metabolites is possible (Garcia-Galan et al., 2008). Therefore, it is hypothesized that the greater amount of SDI is degraded and not adsorbed or transformed to a known metabolite. In line with this, Spielmeyer et al. (2014) reported that in biogas plants, contents of certain antibiotics are higher in the input in comparison to the output. According to this, anaerobic digestion is a potential process to reduce the antibiotic content (Fig. 3). Nevertheless, particular attention must be given to the residues still present in the digestate.

3.5. Biogas production using T. pannonicum as substrate with different SDI concentrations

The specific biogas yield (SBY) depends on the plant part used as substrate (p-value 0.0080) and on the SDI content (p-value 0.0035) (Fig. 4). Both SBY and specific methane yield (SMY) are highest using shoots as substrate of the plants cultivated at 5 mg·L^{-1} SDI with a mean value of 534 ± 24 mL·g^{-1} of VS and 318 ± 15 mL·g^{-1} of VS, respectively. A similar result is obtained using roots as substrate of the plants grown at 10 mg·L^{-1} SDI, with biogas and methane yields of 501 ± 15 mL·g^{-1} of VS and 317 ± 10 mL·g^{-1} of VS, respectively. The lowest yield is obtained by the roots of the control group (plants grown with no SDI), with a biogas yield of 370 ± 12 mL·g^{-1} of VS and methane yield of 217 ± 7 mL·g^{-1} of VS (Fig. 4a). Based on these results, roots and shoots can be used as substrate for biogas production with high SBY and SMY in comparison to other crops (Turcios et al., 2016a, b), avoiding competition for freshwater resources and arable land with food crops. Nevertheless, an optimum biogas yield is achieved with certain SDI contents in the plant material. This could be due to the interaction of SDI with other organic compounds, the enhancement of some organic constituents in the plant tissues due to the stress caused by the antibiotic, or due to the antibiotic itself. To test this assumption, substrate obtained from the plants cultivated in the absence of SDI was used and SDI was added directly to the anaerobic reactors to obtain concentrations of 100 ng·mL^{-1} and 500 ng·mL^{-1}. According to the results, although there are no significant differences between treatments, an optimal SBY is achieved at 100 ng·mL^{-1} SDI with a mean value of 516 ± 25 mL·g^{-1} of VS, and a SMY of 339 ± 17 mL·g^{-1} of VS (Fig. 4b). Based on these findings, it can be assured that SDI at lower concentrations than 500 ng·mL^{-1} an enhancement of biogas production can occur, but at higher concentrations SDI can be toxic for methanogenic microorganisms.

4. Conclusions

Tripolium pannonicum has a high potential as biofilter to uptake the antimicrobial SDI present in water, and subsequently its biomass can be used for biogas production while simultaneously SDI biodegradation takes place. After five weeks under hydroponic conditions, a decrease up to 34.15 ± 3.76 mg per container is quantified. After anaerobic digestion, using shoots as substrate a SDI decrease of 58% is observed, while using roots a decrease up to 88% is found. The maximum specific biogas yield is obtained using shoots as substrate of the plants grown at 5 mg·L^{-1} with a value of 534 ± 24 mL·g^{-1} VS.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

Financial support of Ariel Turcios by the German Academic Exchange Service (DAAD) and the Universidad de San Carlos de Guatemala (Personal identification number: 91548278 funding programme: ALEGUA (57049520)) is gratefully acknowledged. We would like to thank Dr. Kelim Vano Herrera and Yvana Glasenapp for their valuable collaboration, the Institute for Sanitary Engineering and Waste Management, Leibniz University Hannover, and Yvonne Leye, for taking care of the plants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.08.047.

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Appendices

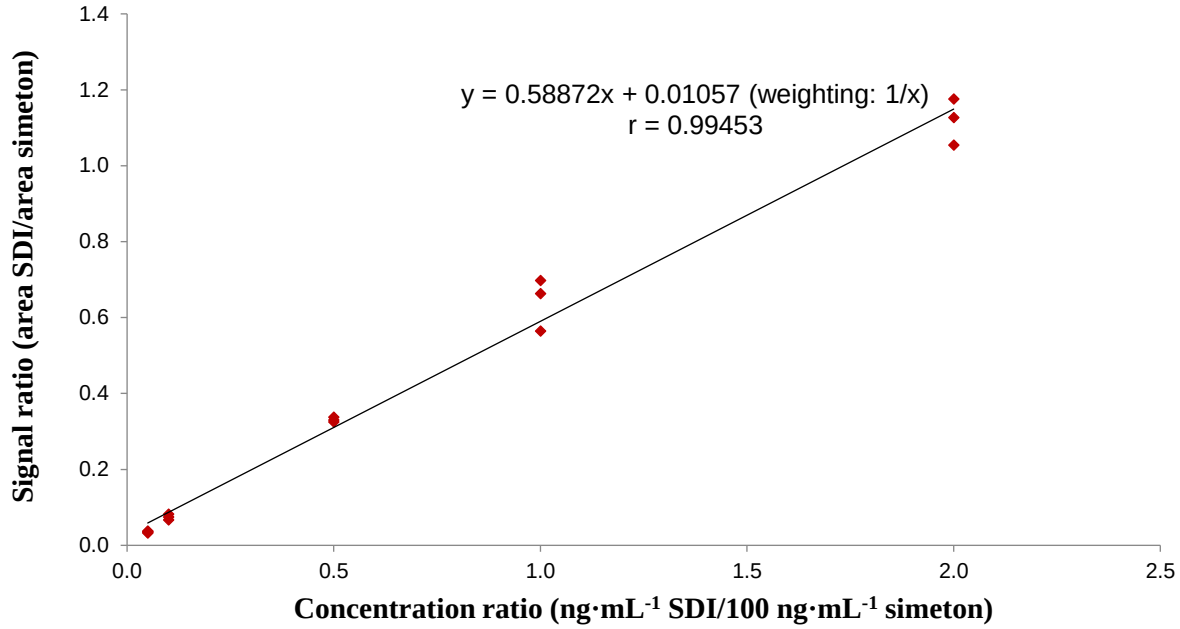

Fig. 1.A. Calibration curve for SDI with simeton as internal standard.

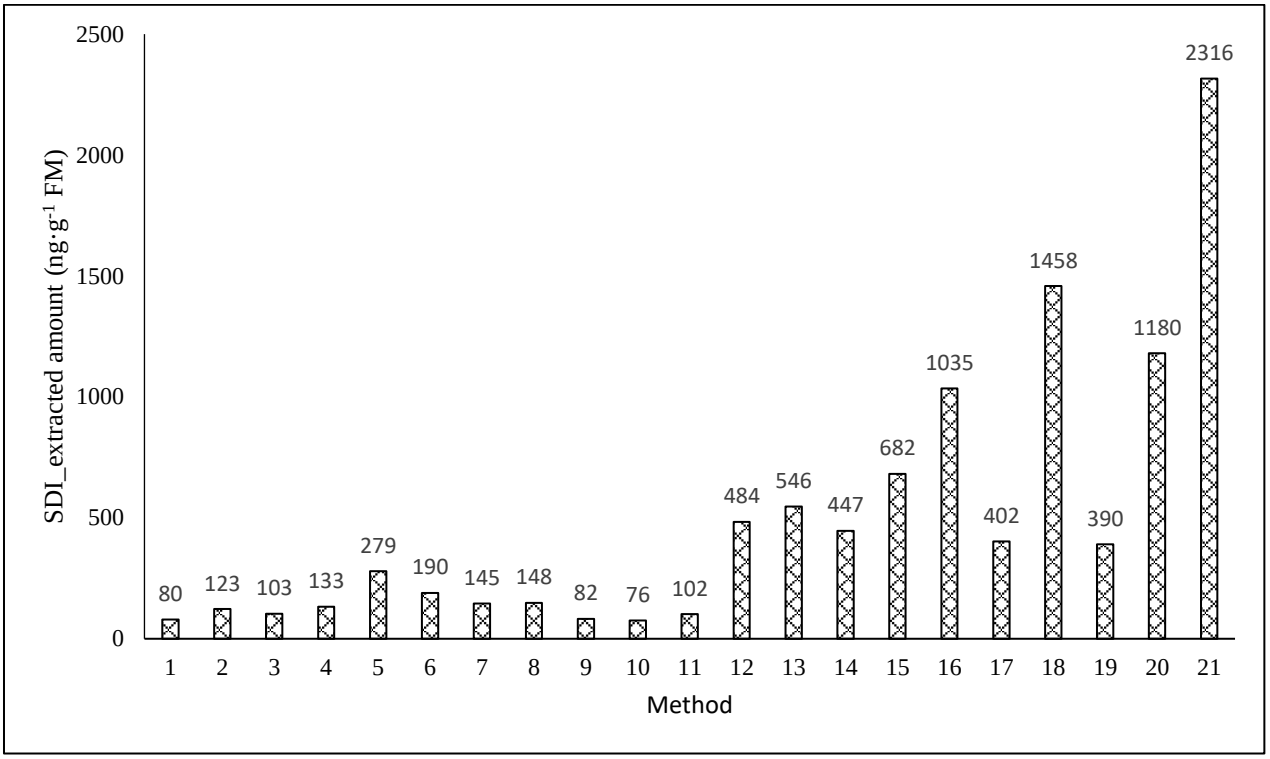

Fig. A.2. Methods used for optimizing the extraction of sulfadimidine from the plant tissues

SDI: Sulfadimidine; ng·g^{-1} FM: nanogram per gram fresh matter. 100 mg ground plant material was weighted in 2 mL reaction tubes and the following methods were applied: **Method 1:** 800 µL MeOH (0.5 M HCl) and 400 µL water (1% ascorbic acid + 0.415% EDTA) was added, sonicated for 10 min, then the samples were centrifuged at 10000 rpm, the supernatant was transferred to a new 2 mL reaction tube, then the pellets were extracted again with 400 µL MeOH, vortexed for 10 min, centrifuged for 5 min at 10,000 rpm, supernatant was taken out and pellets extracted again with 400 µL MeOH. Supernatants were pooled and analyzed by LC-MS. **Method 2:** The same procedure as Method 1 but instead of the bath sonicator (Bioruptor^{TM} Twin, Liège, Belgium), vortex for 10 min was used. **Method 3**: The same procedure as Method 2 but after pooling the supernatants, Solid Phase Extraction was carried out (Oasis HLB 3 cm^3 60 mg, Waters Corporation, Milford, Massachusetts USA). **Method 4**: SDI was extracted with 1000 µL MeOH (0.5 M HCl), vortexed for 10 min, centrifuged at 10000 rpm for 5 min, the supernatant was collected, then the pellets were extracted again with 200 µL acetone and 800 µL MeOH, vortexed for 10 min, centrifuged at 10,000 rpm for 5 min. The supernatants were combined and dried using Speed Vac. Residue was resuspended in 1 mL of methanol:ultrapure water (80:20) and defatted with 1 mL of hexane three times. Hexane was removed (the upper layer) each time after the liquid−liquid partitioning. **Method 5**: Alkaline-acid hydrolysis was used. 230 µL MeOH with 10% acetic acid was added, shaken for 1 h at 1,000 rpm (Eppendorf® Thermomixer Compact, short: thermomixer), then 77 µL H_2O, 577 µL water (1% ascorbic acid and 0.415% EDTA) and 192 µL NaOH (10 M) was added. The samples were shaken for 16 h at 1,000 rpm. Then the pH was adjusted to 2 with concentrated HCl. 308 µL ethyl acetate was added, vortexed and centrifuged, the organic layer was collected (this step was done 3 times). Then the acid hydrolysis was performed adding 100 µL of concentrated HCl and carried out in the same manner as the alkaline hydrolysis. Organic and inorganic layers were dried separately using Speed Vac (Concentrator plus, Eppendorf AG, Hamburg, Germany). Samples were resuspended in 1 mL MeOH:H_2O (80:20) and analyzed separately. **Method 6**: Same procedure as method 5 was carried out but using only alkaline hydrolysis. **Method 7**: A similar procedure as method 5 was carried out with some changes: 100 µL NaOH (10 M) was used instead of 192 µL. Extraction was carried out for 2 h. 150 µL of concentrated HCl was used to adjust the pH. Then 50 µL of concentrated HCl was used for acid hydrolysis. **Method 8**: A similar procedure as method 5 was carried out with some changes: 50 µL NaOH (10 M) was used instead of 192 µL. Extraction was carried out for 2 h. 50 µL of HCl was used to adjust the pH to 3. Then 50 µL of concentrated HCl was used for acid hydrolysis. **Method 9**: A similar procedure as method 5 was carried out with some changes: 500 µL MeOH was added (instead of 230 µL), 50 µL NaOH (10 M) was used instead of 192 µL. Extraction was carried out for 2 h. 25 µL of HCl was used to adjust the pH to 4.5. Then 50 µL of concentrated HCl was used for acid hydrolysis. **Method 10**: A similar procedure as method 5 was carried out with some changes: 50 µL NaOH (10 M) was used instead of 192 µL. Extraction was carried out for 16 h. 25 µL of HCl was used to adjust the pH to 4.5. Then 50 µL of concentrated HCl was used for acid hydrolysis. **Method 11**: A similar procedure as method 5 was carried out with some changes: no NaOH (10 M) was used (only acid hydrolysis was performed). Extraction was carried out for 2 h. 25 µL of concentrated HCl was used to adjust the pH. Then 50 µL of concentrated HCl was used for acid hydrolysis. **Method 12**: A similar procedure as method 5 was carried out with some changes: 50 µL NaOH (10 M) was used instead of 192 µL. Extraction was carried out for 2 h. 25 µL of HCl was used to adjust the pH. Then 50 µL of concentrated HCl was used for acid hydrolysis and then the sample was heated at 85°C for 30 min. **Method 13**: 1000 µL of extraction buffer (1% formic acid in MeOH v/v) was added, sample was shaken at 1000 rpm for 3 h (thermomixer), then 500 µL MeOH (5% HCl v/v) was added, finally sample

was shaken and heated at 85 °C for 30 min (thermomixer). Sample was centrifuged at 10000 rpm for 5 min. Supernatant was diluted (1:4) in 80% MeOH and analyzed. **Method 14**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 2 h (thermomixer) instead of 3 h, 1000 µL MeOH (1% HCl v/v) was added instead of 500 µL (5% HCl). **Method 15**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 2 h (thermomixer) instead of 3 h, 1000 µL MeOH (5% HCl v/v) was added instead of 500 µL. **Method 16**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 2 h (thermomixer) instead of 3 h, 1000 µL MeOH (10% HCl v/v) was added instead of 500 µL (5% HCl). **Method 17**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 2 h (thermomixer) instead of 3 h, 800 µL MeOH (5% HCl v/v) and 200 µL MeOH (1% ascorbic acid + 0.415% EDTA) was added instead of only 500 µL (5% HCl). **Method 18**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 2 h (thermomixer) instead of 3 h, 1000 µL MeOH (5% HCl v/v) was added instead of 500 µL (5% HCl) and sample was heated at 85°C for 60 min instead of 30 min. **Method 19**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 1 h (thermomixer) instead of 3 h, 1000 µL MeOH (5% HCl v/v) was added instead of 500 µL (5% HCl) and sample was heated at 85°C for only 15 min instead of 30 min. **Method 20**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 16 h (thermomixer) instead of 3 h, 1000 µL MeOH (5% HCl v/v) was added instead of 500 µL (5% HCl). **Method 21**: A similar procedure as method 13 was carried out with some changes: sample was shaken at 1000 rpm for 4 h (thermomixer) instead of 3 h, 1000 µL MeOH (10% HCl v/v) was added instead of 500 µL (5% HCl) and then sample was heated at 85°C for 40 min instead of 30 min.

Table A.1. Precision and accuracy for quantification of sulfadimidine.

Calculated concentration ($ng \cdot mL^{-1}$)								
Standard	**Actual concentration ($ng \cdot mL^{-1}$)**	**Value 1**	**Value 2**	**Value 3**	**mean**	**Bias (%)**	**SD ($ng \cdot mL^{-1}$)**	**CV (%)**
1	5	4.67	4.09	4.52	4.43	-11.40	0.30	6.80
2	10	9.62	12.19	10.97	10.93	9.30	1.29	11.78
3	50	53.44	54.29	55.59	54.44	8.88	1.09	1.99
4	100	94.19	110.90	116.70	107.26	7.26	11.70	10.90
5	200	177.40	189.70	198.00	188.37	-5.82	10.37	5.50

SD = standard deviation (n=3); CV = coefficient of variation

Bias (%) = [(determined mean value − true value) / true value] × 100).

Table A.2. Analysis of variance and Tukey test for the variable sulfadimidine content in the plant tissues.

Analysis of variance

S.V.	SS	df	MS	F	*p*-value
Model.	320.67	7	45.81	9.91	0.0001
culture medium	86.26	1	86.26	18.67	0.0005
plant part	199.49	3	66.50	14.39	0.0001
culture medium*plan part	34.92	3	11.64	2.52	0.0948
Error	73.93	16	4.62		
Total	394.61	23			

Test:Tukey Alpha:=0.05 LSD:=1.86035

Error: 4.6207 df: 16 S.E.: 0.62

culture medium (mg·L^{-1})	Means	n		
10.00	6.09	12	A	
5.00	2.30	12		B

Means with a common letter are not significantly different (p > 0.05)

Test:Tukey Alpha:=0.05 LSD:=3.55069

Error: 4.6207 df: 16 S.E.: 0.88

plant part	Means	n			
st	8.61	6	A		
ol	4.10	6		B	
s	3.52	6		B	C
yl	0.55	6			C

Means with a common letter are not significantly different (p > 0.05)

Test:Tukey Alpha:=0.05 LSD:=6.07650

Error: 4.6207 df: 16 S.E.: 1.24

culture medium (mg·L^{-1})	plant part	Means	n			
10.00	st	12.04	3	A		
10.00	ol	6.73	3	A	B	
5.00	st	5.18	3		B	C
10.00	s	4.72	3		B	C
5.00	s	2.32	3		B	C
5.00	ol	1.48	3		B	C
10.00	yl	0.87	3		B	C
5.00	yl	0.22	3			C

Means with a common letter are not significantly different (p > 0.05)

S.V. = source of variation. SS = sum of squares. df = degrees of freedom. S.E. = standard error. MS = mean square. LSD = least significant difference. n = number of replicates

5 = 5 mg·L^{-1} sulfadimidine in the growth media; 10 = 10 mg·L^{-1} sulfadimidine in the growth media; yl = young leaves; ol = old leaves; st = stem; s = shoots. Values represent means of 3 technical replicates per treatment in µg·g^{-1} FM.

Chapter 6

General discussion

Biomass yield of halophytes cultivated under different salt concentrations

In accordance with the results obtained (chapter 3), the biomass yield depends on the salt concentration in the growth medium and on the plant species, although the agronomic crop management and environmental factors may also affect the biomass productivity. The greatest biomass production of *T. pannonicum* is obtained from the plants cultivated under non-saline conditions with a yield of 6.90 $kg \cdot m^{-2}$ FM (0.57 $kg \cdot m^{-2}$ DM) and then decreases progressively at higher salinities to reach 294 $g \cdot m^{-2}$ FM (42 $g \cdot m^{-2}$ DM) at 45 $g \cdot L^{-1}$ NaCl. However, plants cultivated in the presence of artificial seawater produce more biomass at 15 $g \cdot L^{-1}$ sea salt, possibly due to the microelement content in this type of salt that could enhance the plant growth. In the case of the species *C. quinoa* (chapter 4), the highest biomass yield is obtained with a salinity of 10 $g \cdot L^{-1}$ NaCl with a value of 8.18 $kg \cdot m^{-2}$ FM, while the greatest dry biomass yield is produced under non-saline conditions (1.04 $kg \cdot m^{-2}$ DM) and then it decreases progressively at higher salinities. This biomass decrease at high salinities is due to the plant's response to salinity stress. Halophytes have different mechanisms to deal with salinity, which depend on each species. These plants use the controlled uptake of Na^+ into cell vacuoles to drive water into the plant against a low external water potential (Glenn et al., 1999). As a result of the osmoregulation process, the water content in the cells may vary, and therefore the dry and fresh weight can be non-proportional under different salinity treatments. For example, the water content in *T. pannonicum* progressively decreases from 91.77% under non-saline conditions to 85.70% at 45 $g \cdot L^{-1}$ NaCl (chapter 3), while in *C. quinoa* the highest water content is found in the plants cultivated at 10 $g \cdot L^{-1}$ NaCl with a value of 89.37%, and the lowest is found in the control group (non-saline conditions) with a value of 84.09% (chapter 4). This explains the high fresh biomass yield from *C. quinoa* at 10 $g \cdot L^{-1}$ NaCl. In addition to this water-content change as a response to salt-stress, accumulation of organic compounds and inorganic ions for osmotic adjustment may occur, therefore the total solids in the plant tissue can differ between different saline conditions.

High salinities in the culture medium cause a decrease in biomass. Although halophytes are able to uptake sodium, energy is needed for Na^+ transport within the plant. In addition, antagonistic effects between elements could occur, therefore the plant growth at high salinities may be adversely affected. Below the toxic levels, it is much more advantageous for the plant to use sodium as a metabolically cheap osmoticum for the purposes of osmotic adjustment (Hariadi et al., 2011). On this basis, for most halophytes there is a direct relationship between

salt concentration in the growth medium and Na^+ content in the plant tissue (Hariadi et al., 2011; Turcios et al., 2016a; Turcios et al., 2016b). However, Ca^{2+} and K^+ uptake may have an inverse relationship with salinity since both cations may compete with Na^+, although it also depends on the species. For example, the K content in the plant tissue of *T. pannonicum* decreases at high salinity, while in *C. quinoa*, an increase is observed under saline conditions. In line with this, Hariadi et al. (2011) found that increased external NaCl concentrations caused a progressive increase in sap K^+ in old leaves, with a strong positive correlation between sap K^+ and Na^+ content in old leaves. The authors stated that >95% of cell osmotic turgor in old leaves of *C. quinoa*, and between 80% and 100% of cell turgor in young leaves may be adjusted by means of accumulation of Na^+ and K^+ only (Hariadi et al., 2011). Some studies have also shown that physiologically relevant concentrations of free amino acids and glycinebetaine are efficient in controlling K^+ transport across the plasma membrane of barley (Cuin and Shabala, 2005; Cuin and Shabala, 2007), which might contribute to the accumulation of potassium in the plant tissue.

Potential use of halophytes for biogas production

The composition and quality of biomass vary depending on the crop management, climatic conditions, and genetic characteristics of each species, among others. Biogas and methane yields depend also on the biomass composition used as substrate, including the salt content (Turcios et al., 2016a; Turcios et al., 2016b). On this basis, specific biogas yield oscillates between 430 $mL \cdot g^{-1}$ VS (271 $mL \cdot g^{-1}$ VS methane) and 554 $mL \cdot g^{-1}$ VS (347 $mL \cdot g^{-1}$ VS methane) using biomass of *T. pannonicum* (chapter 3), from 342 $mL \cdot g^{-1}$ VS (220 $mL \cdot g^{-1}$ VS methane) to 470 $mL \cdot g^{-1}$ VS (305 $mL \cdot g^{-1}$ VS methane) using biomass of *C. quinoa* (chapter 4), and 499 $mL \cdot g^{-1}$ VS (309 $mL \cdot g^{-1}$ VS methane) using biomass of *Salicornia* spp (Turcios et al., 2015). Since halophytes are able to uptake salt from the growth medium, the effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *T. pannonicum* was investigated (Chapter 3). According to the results, the higher the salt concentration in the culture medium is, the greater the amount of sodium in the plant tissue. Biomass of plants cultivated in the presence of salt (30 $g \cdot L^{-1}$ NaCl) produced the greatest amount of methane, but at higher salt concentrations in the plant cultivation medium the methane yield decreased. This enhancement of methane using salty biomass it not because of the sodium content, but the content of certain organic compounds as discussed below. A better quality of substrate of the plants grown under saline conditions counteracts the negative effects of sodium during anaerobic digestion. Using

substrate of plants cultivated under non-saline conditions (control) and adding different amounts of NaCl to the reactors, the specific methane and biogas yield decreases, therefore using the same substrate and by varying the amount of salt can be observed that it inhibits the production of biogas (Chapter 3, Fig. 4). However, the presence of low Na^+ concentrations is essential for the methanogenic archaea, presumably because it is important for the formation of ATP or the oxidation of NADH (Appels et al., 2008), but high concentration of this element has negative effects on anaerobic digestion. In high –salt content substrates, sodium apparently is the main element that inhibits the production of biogas (Chen et al., 2008). Divalent cations such as Ca^{2+} and Mg^{2+} are important for floc strength, which could enhance bacterial growth and symbiosis between microorganisms, but high concentrations of monovalent cations, like Na^+, may cause sludge deflocculation (Sudhir et al., 1999). In addition, a high concentration of Na^+ causes microbial cells to dehydrate due to osmotic pressure. In agreement with this, based on a comparison of sodium sulfate, sodium nitrate and sodium chloride, Sistrom (1960) reported that Na^+ is the main ion responsible in affecting the growth of *Rhodopseudomonas sphaeroides*. To address this inhibitory effects, an adaptation of the bacteria and archaea populations to the salinity is possible (Chapter 3). According to the results, the highest methane yield is obtained by using a salt-adapted inoculum (2.181 $g \cdot L^{-1}$ Na^+) and biomass of *T. pannonicum* as substrate with a yield of 252 $mL \cdot g^{-1}$ VS, while using a non-adapted inoculum a methane production of 226 $mL \cdot g^{-1}$ VS is observed. By using the non-adapted inoculum and adding 6.36 $g \cdot L^{-1}$ NaCl to the anaerobic reactors the specific methane yield fell to 199 $mL \cdot g^{-1}$ VS. In line with this, Feijoo et al. (1995) also observed a tolerance to sodium in the sludge obtained from the digesters treating high saline wastewaters and increasing the methanisation of volatile fatty acids due to the microbial adaptation to sodium. They also reported that after 40 days of digestion, using two different sludges (subjected to 6.9 and 21 .5 $g \cdot L^{-1}$ Na^+, respectively) increased the relative methanogenic activity from 0% to about 45% of the blank activity (Feijoo et al., 1995).

Biogas and methane yields also depend on the plant species and biomass quality used as substrate. This led us to investigate the potential use of the facultative halophyte *Chenopodium quinoa* Willd. as substrate for biogas production cultivated with different concentrations of sodium chloride under hydroponic conditions (Chapter 4). This halophyte has a high potential for biogas production with yields up to 470 $mL \cdot g^{-1}$ VS at 20 $g \cdot L^{-1}$ NaCl in the growth medium, however, salinity has an influence on biomass composition and therefore on biogas yield (Fig. 1).

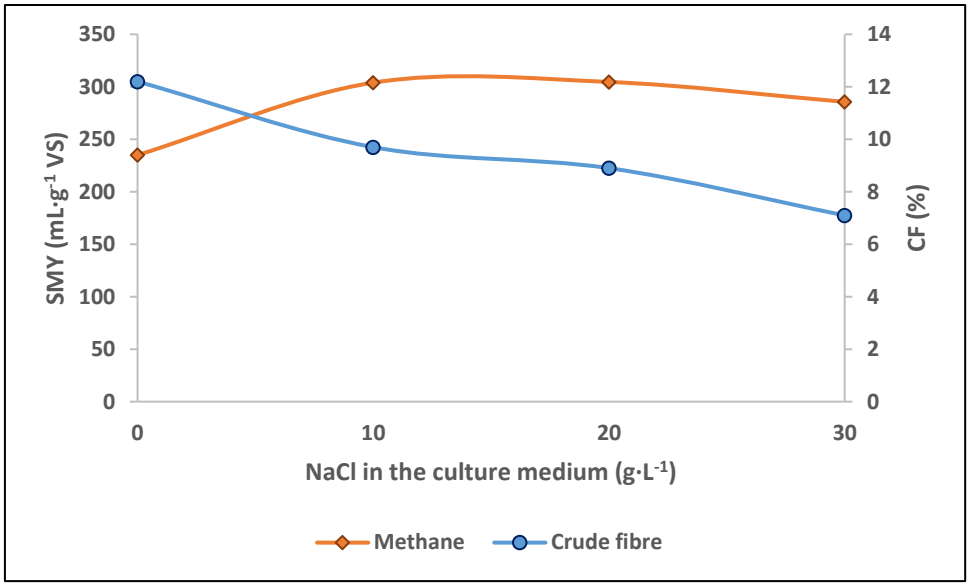

Fig. 1. Relationship between salinity in the growing medium of *C. quinoa*, crude fibre in the plant tissue and methane production. SMY = specific methane yield; CF = crude fibre

The biomass composition also depends on the plant age. In plants cultivated for five weeks under hydroponic conditions, the salt concentration in the culture medium is directly proportional to the CA and HCEL content in the plant material, while CP, CF, ADF, NDF, CH, CEL and starch have an inverse relationship (Chapter 4, Fig. 2). Plants cultivated for 12 weeks under hydroponic conditions, show higher contents of CA and CP at high salinities, whereas ADL is higher under non-saline conditions. According to Rath et al. (2013), CL and HCEL are positively correlated with biogas yield, while ADL and CH have a negative influence on biogas production. This indicates that biomass from plants cultivated under saline conditions would produce greater biogas yield, since HCEL and CL are higher in comparison to the control (non-saline conditions). Furthermore, in the species *T. pannonicum* CA, CL and HCEL also have a positive relationship with the salt content in the culture medium where plants were cultivated (Chapter 3), whereas CP, CF, ADF, NDF, ADL, and cellulose have an inverse relationship. The results also confirm that biomass of *T. pannonicum* cultivated under saline conditions produces the greater methane yield. In both crops, CL and HCEL are found in an increased amount in comparison to the control which have favored biogas and methane production. Lipids (CL) are the most effective sources of storage energy in living beings and they also play an important role in the tolerance to several physiological stressors in a variety of organisms. The mechanism to avoid dehydration is based on phospholipid bilayers, which are stabilized during water stress by sugars. Unsaturation of fatty acids also counteracts water or salt stress (Parida and Das, 2004). In both species the cellulose content is lower in the plants cultivated in the presence of salt. Cellulose is a polymer, with insoluble and crystalline microfibrils, which are highly

resistant to hydrolysis. Hydrolysis is considered the rate-limiting step in digesters fed with a high cellulosic substrate; consequently, substrate with low cellulose content would improve the biodegradation of organic matter. In addition, changes in some chemical elements in plants treated with different salinity concentrations are also observed, which may positively or negatively affect the biogas digestion process. For example, under saline conditions the content of both K and Ca decreases in the plant tissue of *T. pannonicum* and the content of Na increases, while in *C. quinoa* the quantity of both K and Na increases under saline conditions but Ca decreases. These changes in the content of elements between both species could be due to defense mechanisms against salt-stress, which depend on the species. For example, *C. quinoa* accumulates K^+ and Na^+ for osmotic adjustment under saline conditions (Hariadi et al., 2011) but Ca^{2+} decreases, presumable due to the competition between both cations Ca^{2+} and Na^+, of which Ca^{2+} has a higher molecular weight being easier for the plants to uptake Na^+.

Methane yields obtained from the halophytes *T. pannonicum*, *C. quinoa* and *Salicornia* spp. are comparable to that obtained from other energy crops, ranging from 271 $mL \cdot g^{-1}$ VS to 347 $mL \cdot g^{-1}$ VS in *T. pannonicum*, from 220 $mL \cdot g^{-1}$ VS to 305 $mL \cdot g^{-1}$ VS in *C. quinoa*, and 309 $mL \cdot g^{-1}$ VS using biomass of *Salicornia*. For example, Amon et al. (2007) reported that methane yields from late ripening maize varieties range between 312 $mL \cdot g^{-1}$ VS and 365 $mL \cdot g^{-1}$ VS (milk ripeness) and from 268 $mL \cdot g^{-1}$ VS to 286 $mL \cdot g^{-1}$ VS (full ripeness). Based on these findings, biomass from halophyte plants do not negatively affect the biogas yield, on the contrary, this substrate enhances biogas production due to the enhance of some organic compounds as previously discussed, although this also depends on the plant species, environmental conditions and agronomic crop management. On the other hand, the energy productivity needs also to be taken into account, since at high salt concentrations the crops' yield per unit area decreases, therefore the methane productivity would also decrease. For example, *C. quinoa* cultivated for five weeks under non-saline conditions (control) and at 10 $g \cdot L^{-1}$ NaCl the methane productivity decreases with no significant differences from 205 $L_N \cdot m^{-2}$ CH_4 to 194 $L_N \cdot m^{-2}$ CH_4 respectively, but at higher salinities the productivity drops significantly up to 48 $L_N \cdot m^{-2}$ CH_4 at 30 $g \cdot L^{-1}$ NaCl. However, using crop residues of *C. quinoa* cultivated for twelve weeks under hydroponic conditions, the methane productivity increases with no significant differences from 211 $L_N \cdot m^{-2}$ CH_4 under non-saline conditions to 228 $L_N \cdot m^{-2}$ CH_4 at 10 $g \cdot L^{-1}$ NaCl and then it decreases significantly to 119 $L_N \cdot m^{-2}$ CH_4 at 20 $g \cdot L^{-1}$ NaCl in the growth medium (Chapter 4, Table 3). This productivity is directly related to the biomass yield, therefore, from a productivity point of view, it is not recommended to use salinities higher than 10 $g \cdot L^{-1}$ NaCl or higher than 15 $g \cdot L^{-1}$ sea salt.

Biofiltration potential of halophyte plants

Halophyte plants have attracted the attention of the international scientific community due to their ability to grow in saline environments and also for their phytoremediation potential. Different halophyte filter beds in constructed or natural wetlands for wastewater treatment have been reported (Buhmann and Papenbrock, 2013; De Lange et al., 2013). As described earlier, each of the treatment methods has its specific shortcomings. In this regard, halophyte plants are not able to remove all pollutants from wastewater and the uptake efficiency depends on a number of factors such as pollutant concentration, physical-chemical characteristics of the pollutants, hydraulic retention time, the growth media, environmental condition and plant species, among others. For example, the uptake of nitrate and phosphate by the facultative halophyte *C. quinoa* is higher in comparison to the halophyte *T. pannonicum* (Table 1 and Table 2). Moreover, the uptake efficiency also depends on the salt concentration in the growth media, since the biomass yield decreases at high salinities.

Table 1. Nutrient decrease in the growth medium of *C. quinoa* during 5 weeks of cultivation with different concentrations of NaCl.

Salinity treatment	**0 g·L^{-1} NaCl**	**10 g·L^{-1} NaCl**	**20 g·L^{-1} NaCl**	**30 g·L^{-1} NaCl**	***p*-value**
Variables					
$\Delta c.NO_3$-N [mg·L^{-1}] ± SE	362.72 ±25.10^{a}	370.16 ±6.53^{a}	214.81 ±17.57^{b}	28.91 ±8.06^{c}	<0.0001
$\Delta c.PO_4$-P [mg·L^{-1}] ± SE	100.97 ±7.06^{a}	105.17 ±2.85^{a}	71.06 ±4.21^{b}	21.24 ±1.68^{c}	<0.0001
$\Delta c.NH_4$-N [mg·L^{-1}] ± SE	72.21 ±0.69^{a}	69.21 ±1.96^{a}	55.67 ±7.95^{ab}	27.05 ±10.72^{b}	0.0055

$\Delta c.NO_3$ = decrease of nitrates during the experiment; $\Delta c.PO_4$ = decrease of phosphates during the experiment; $\Delta c.NH_4$ = decrease of ammonium during the experiment. Eight plants were cultivated in a total volume of 13.5 L of culture medium. Values represent mean ± SE values of 3 technical replicates per treatment. Significant differences (p -value≤ 0.05) between salinity treatments are indicated by different letters within the same row ($^{a, b, c}$)

Table 2. Nutrient decrease in the growth medium of *T. pannonicum* during 5 weeks of cultivation with different concentrations of NaCl.

Salinity treatment	**0 g·L^{-1} NaCl**	**15 g·L^{-1} NaCl**	**30 g·L^{-1} NaCl**	**45 g·L^{-1} NaCl**	***p*-value**
Variables					
$\Delta c.NO_3$-N [mg·L^{-1}] ± SE	216.51±6.63^{a}	168.38±15.76^{b}	26.73±4.57^{c}	15.79±0.70^{c}	<0.0001
$\Delta c.PO_4$-P [mg·L^{-1}] ± SE	67.19±2.88^{a}	46.91±8.63^{a}	9.58±1.51^{b}	4.51±1.21^{b}	<0.0001
$\Delta c.NH_4$-N [mg·L^{-1}] ± SE	40.65±1.44^{a}	41.34±0.21^{a}	38.33±1.30^{a}	15.93±2.42^{b}	<0.0001

$\Delta c.NO_3$ = decrease of nitrates during the experiment; $\Delta c.PO_4$ = decrease of phosphates during the experiment; $\Delta c.NH_4$ = decrease of ammonium during the experiment. Eight plants were cultivated in a total volume of 13.5 L of culture medium. Values represent mean ± SE values of 3 technical replicates per treatment. Significant differences (p -value≤ 0.05) between salinity treatments are indicated by different letters within the same row ($^{a, b, c}$)

Halophytes are also able to uptake salt from the growth media, therefore they have the potential for soil or water desalination, and also offer a great potential for the decontamination of heavy metals (Manousaki and Kalogerakis, 2011). In addition, *T. pannonicum* is able to uptake the antibiotic sulfadimidine (SDI) (This is discussed in more detail below). All these advantages make halophyte plants more interesting from both an economic and environmental point of view, although some glycophytes may be used for treating non-saline wastewater. For example, hyacinth (*Eichhornia crassipes*), pennywort (*Hydrocotyle umbellata*) and water lettuce (*Pistia stratiotes*), among other glycophytes have been used for treating wastewater. Nevertheless, salinity appears to be the principal reason for growth inhibition, with toxic effects in water lettuce and water hyacinth when these plants are exposed to diluted seawater with salt concentrations of 1.66 $g \cdot kg^{-1}$ and 2.50 $g \cdot kg^{-1}$, respectively (Sooknah and Wilkie, 2004). The same authors also reported that the combined effect of an initial Na^+ concentration of 52.4 $mg \cdot L^{-1}$, 130 $mg \cdot L^{-1}$ NH_4–N, and the presence of uncharacterized soluble compounds likely stressed the plants and limited their ability to grow and develop (Sooknah and Wilkie, 2004). Most wastewater effluents contain dissolved salts, for instance, concentrations ranging from 10 $g \cdot L^{-1}$ up to 71 $g \cdot L^{-1}$ in some effluents treated anaerobically have been reported (Lefebvre and Moletta, 2006). Therefore, glycophyte plants are not recommended for treating wastewater with high salt concentration. Some salt-tolerant grasses such as common reed (*Phragmites australis*) has been also reported for treating wastewater in constructed wetlands (Srivastava et al., 2014). This species is able to grow in a salinity to around 22 $g \cdot L^{-1}$, though the relative growth rates of rhizome-grown plants on a wet weight basis is optimum at 5 $g \cdot L^{-1}$ salinity (Lissner and Schierup, 1997). Politeo et al. (2011) reported that in two full-scale horizontal subsurface flow constructed wetlands, total nitrogen and total phosphorus removal efficiencies up to 48% and 38% were achieved by *P. australis*, respectively. In addition, under optimal climatic conditions, this species is able to produce fresh biomass yield up to 105 $t \cdot ha^{-1}$ annually (Politeo et al., 2011). Based on this, *P. australis* has also a high potential as biofilter.

Occurrence of antibiotics in the environment and their removal from water resources

Pharmaceutical compounds dissolved in water have also caused concern since these pollutants are discarded to the environment and are not completely removed in WWTPs. For this reason, a wide range of antibiotics have been found in surface water, groundwater and even in drinking water, and their presence may promote antibiotic resistance in bacteria. The development of antibiotic resistance has led to a reduction in the effectiveness of antibiotics for the treatment of infectious diseases, and this is a major threat to human health globally (Murdoch, 2015). Antibiotics most commonly found in the water are tetracycline, oxytetracycline,

chlortetracycline, sulfonamides, trimethoprim, amoxicillin, clarithromycin, ampicillin, penicillin, ciprofloxacin, erythromycin, streptomycin, norfloxacin, ofloxacin, cefaclor, cephalexin, lincomycin, spiramycin, among others (Fatta-Kassinos et al., 2011; Miège et al., 2009; Murdoch, 2015; Nikolaou et al., 2007). The concentrations of these antibiotics in surface waters and the effluent from WWTPs have been shown to lie in the $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ range (Hirsch et al., 1999; Nikolaou et al., 2007), although concentrations up to 9 $mg \cdot kg^{-1}$ in slurry have been reported (Spielmeyer et al., 2014). However, the concentration and the type of antibiotic may vary depending on the country and the source point. For example, in England, Germany and Austria some pharmaceutical products are used in quantities of more than 100 tons a year (Nikolaou et al., 2007), and unfortunately, most of these antibiotics are not very well removed by activated sludge in WWTPs. In the case of trimethoprim, for example, Deblonde et al. (2011) reported that a removal rate between 40–50% is achieved. Nevertheless, this removal rate in WWTPs depends on many factors such as the treatment method, antibiotic concentration, changes in temperature, solar radiation, retention time, biodegradation, sorption, among others. Based on this, mechanisms of removal do not follow a general rule since it also depends on the physico-chemical properties of the micropollutant, the origin and composition of the wastewater, and the operational parameters of the WWTPs (Cirja et al., 2008). For example, photodegradation is an elimination process that will be less effective during wintertime when solar radiation is minimum. For many compounds, sorption increases as the temperature decreases, while biodegradation is less effective when the temperature decreases (Deblonde et al., 2011). Adsorption takes place due to electrostatic interactions, which is directly proportional to the dissolved concentration of the substance and suspended solids (Cirja et al., 2008).

The use of plants as biofilters to remove organic and inorganic compounds from water have been explored recently and deserve the special attention of the international community (Herklotz et al., 2010; Politeo et al., 2011; Sooknah and Wilkie, 2004; Turcios et al., 2016c). For example, Haase et al. (2015) reported that microalgae cultures of *Parachlorella kessleri* show great potential to withstand carbamazepine as an environmental pollutant, with a decrease in the culture medium up to 44.7% (from an initial concentration of 1 $\mu g \cdot L^{-1}$ to a final concentration of 0.55 $\mu g \cdot L^{-1}$). In this regard, the halophyte *Tripolium pannonicum* was cultivated under hydroponic conditions to study the removal rate of the antibiotic sulfadimidine (SDI) and its biodegradation in the plant tissues and through the anaerobic digestion process (Chapter 5). *Tripolium pannonicum* has a high potential as biofilter, even though the biomass yield decreases at high SDI concentrations. Each antibiotic has its own mechanism of action,

therefore the decrease of biomass could be because SDI interferes with folic acid synthesis via competition with para-aminobenzoic acid metabolism and consequently decrease the amount of folate in the plant (Zhang et al., 2012). It also depends on the plant species since each species, whether aquatic or terrestrial, has its own defense mechanisms to overcome the negative effects. This include selective uptake mechanisms, dissipation of the antibiotic in the rhizosphere or by a release of stress response hormones (Mathews et al., 2013). It should also be taken into consideration that the concentrations used are the highest that can be found in the environment (up to 10 $mg \cdot L^{-1}$), and even at this concentration *T. pannonicum* performs well as biofilter to uptake SDI.

The amount of SDI taken up by the plants depends also on the concentration in the culture medium, which are positively correlated. In the culture medium, with an initial SDI concentration of 65 milligram per container (5 $mg \cdot L^{-1}$), a SDI decrease of 28.25 ± 2.81 milligrams per container (2.17 ± 0.21 $mg \cdot L^{-1}$) is observed, whereas in the plant growth media with an initial concentration of 130 milligram per container (10 $mg \cdot L^{-1}$ SDI), a reduction of 34.15 ± 3.76 milligrams per container (2.63 ± 0.29 $mg \cdot L^{-1}$) is observed. This indicates that a SDI decrease up to 43% is carried out by the plants, depending on the SDI concentration. At higher concentrations, the plant biomass decreases therefore the uptake capacity does not behave in a linear fashion. For example, in the shoots of the plants cultivated at 5 $mg \cdot L^{-1}$ SDI, an amount of 2.32 $\mu g \cdot g^{-1}$ FM of SDI is found, and in those cultivated at 10 $mg \cdot L^{-1}$ SDI, an amount of 4.72 $\mu g \cdot g^{-1}$ FM of SDI is found. However, for the plants grown at 5 $mg \cdot L^{-1}$ SDI, the total amount of SDI quantified in the plant material (including roots and shoots of the 8 plants per container) is of 10.88 mg SDI, while those cultivated at 10 $mg \cdot L^{-1}$ SDI, 10.06 mg SDI is quantified (chapter 5).

Since the amount of SDI taken up by the plants depends on the concentration in the culture medium it indicates that this antibiotic is absorbed and transported in a passive way, without spending extra energy. In addition, the content of SDI within the plant also depends on the plant part. The highest content of SDI is found in the roots followed by the stem. Since the octanol/water partition coefficient (Kow) of SDI is low (log Kow = 0.14), which means that SDI is highly soluble in the aqueous phase, it would be expected to be easily transported via the xylem vessels. This is probably the reason why most SDI is found in roots and stem. According to his study, biodegradation of SDI could take place in the plant tissues. Of the decreased amount in the culture medium, between 29-39% was quantified in the plant material, which means that more than 61% may have been degraded by plants. There might be also significant concern about the undegraded amount of SDI found in the plant material. For this

reason, the plant biomass was used for biogas production through the anaerobic digestion process, while at the same time SDI degradation takes place (Fig. 2).

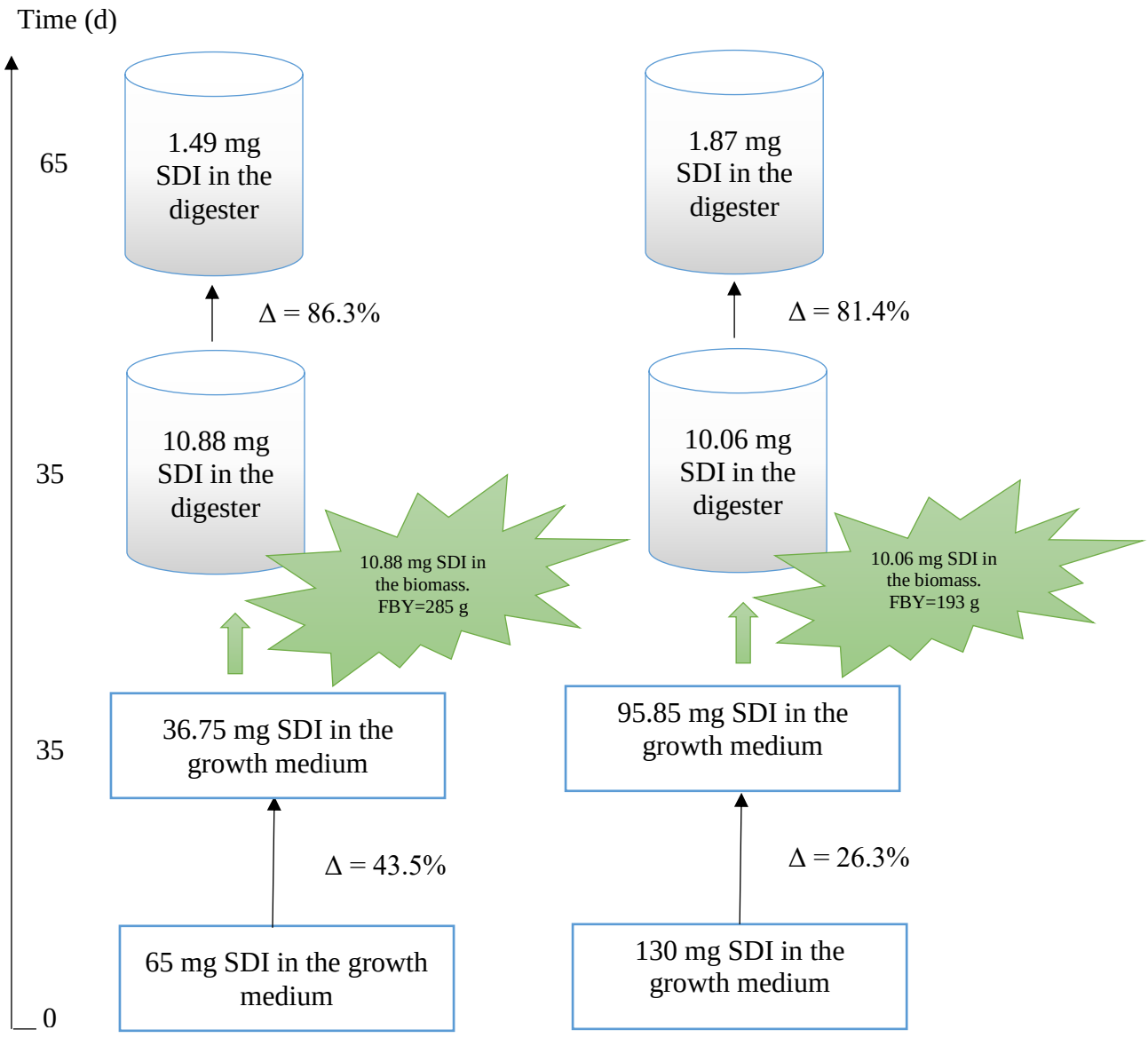

Fig. 2. Uptake and biodegradation of sulfadimidine by the species *T. pannonicum* and its further biodegradation during anaerobic digestion. SDI = sulfadimidine; FBY = fresh biomass yield per container (including shoots and roots); Δ = decrease of sulfadimidine (taking into account the total biomass of roots and shoots). Values represent the mean of three technical replicates per treatment. Each container had 13 L of growth medium.

Based on the difference between the initial and final quantified concentration, a SDI decrease up to 88% during anaerobic digestion is observed. Although a certain amount of SDI may be adsorbed by the anaerobic medium evading its chemical analysis, it is hypothesized that the greater amount of SDI is degraded by microorganisms, mainly because SDI can easily be extracted due to its high water solubility. Furthermore, the same behavior can be observed where SDI was added to the anaerobic reactors and also no matrix effect is observed. According

to Cirja et al. (2008), compounds with log Kow < 2.5, the sorption to sludge is not expected, while log Kow between 2.5 and 4 moderate sorption could occur. Since log Kow for SDI is much lower than these values (0.14), adsorption to inoculum is not expected. In addition, different amounts of SDI were added to the anaerobic medium, the quantification was carried out on the same day and no SDI reduction was observed, so adsorption of SDI may not have occurred. Based on these results, this whole process is very interesting because it is an effective and sustainable system from an economic, social and environmental point of view. Wastewater can be treated with plants acting as biofilter to uptake pollutants and subsequently the biomass plant can be used for renewable energy production where biodegradation of these organic compounds is carried out (Fig. 3).

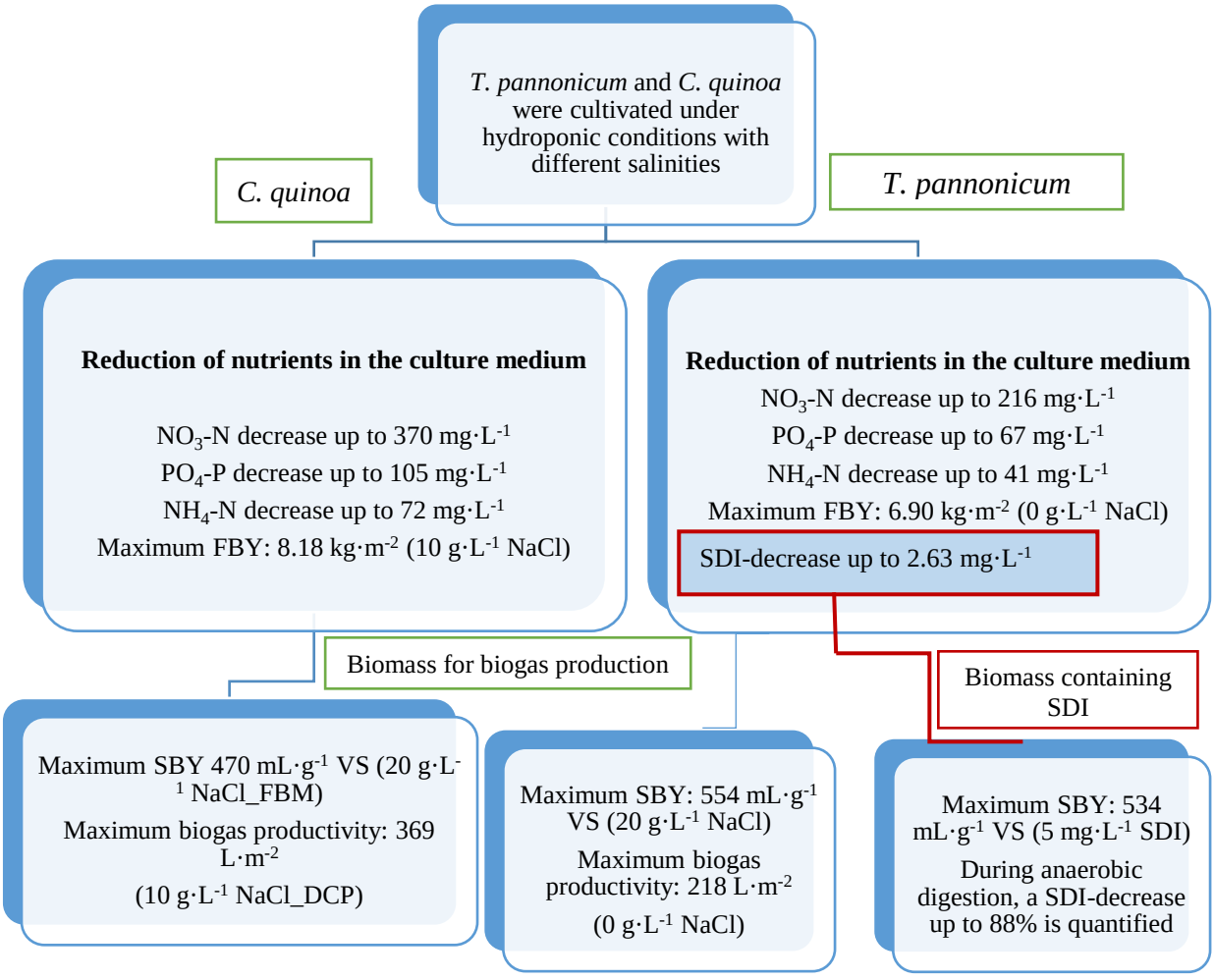

Fig. 3. Summary of the most important results

SBY = specific biogas yield; FBY = fresh biomass yield (plants cultivated for 5 weeks under hydroponic conditions); FBM = fresh biomass (plants cultivated for 5 weeks under hydroponic conditions); DCP = dry crop residues from the plants cultivated for 12 weeks under hydroponic conditions; g·L^{-1} NaCl = gram per liter of sodium chloride in the culture medium; mL·g^{-1} VS = milliliter per gram of volatile solids; SDI = sulfadimidine.

Future implications

Halophyte plants have a promising future, due to the scarcity of water resources and salinization of water and soil. Furthermore, these plants have a high potential for biofiltering salty wastewater by reducing organic and inorganic contaminants such as nitrates, phosphates and pharmaceutical compounds, whilst biomass can be used for renewable energy production. Nevertheless, some other future research tasks are also important. The research of other halophytes which may produce more biomass and therefore the uptake of pollutants would be enhanced is promising. In addition, the biofiltration of other emerging pollutants and their synergic effects on the plant growth should also be investigated in the future.

Conclusions

Tripolium pannonicum which is a salt-tolerant species is capable to grow under high saline conditions up to 45 $g \cdot L^{-1}$ NaCl, although its biomass yield decreases at high salinities. *Chenopodium quinoa* is also capable to grow up to 40 $g \cdot L^{-1}$ NaCl and the highest fresh biomass is obtained with a salt concentration in the culture medium of 10 $g \cdot L^{-1}$ NaCl but biomass decreases at higher salt concentrations.

Biomass of halophytes has a high potential for biogas production. Specific biogas yield ranges between 342 $mL \cdot g^{-1}$ VS (*C. quinoa* grown under non-saline conditions) and 554 $mL \cdot g^{-1}$ VS (*T. pannonicum* grown under saline conditions). Therefore, biomass from these halophytes cultivated in saline environments can be used for biogas production and methane yields are comparable to those obtained from energy crops.

Using biomass of halophytes cultivated under saline conditions does not inhibit methane production, quite the contrary, an enhancement is obtained with salt concentrations in the hydroponic growing medium between 20 and 30 $g \cdot L^{-1}$ NaCl. In addition, a salt adapted inoculum produces a greater amount of biogas using salty biomass in comparison to the non-adapted.

Halophytes, which are salt tolerant plants, are able to uptake organic and inorganic pollutants from water such as nitrates, phosphates, heavy metals, pharmaceutical compounds, among others and, subsequently, their biomass has high potential for renewable energy production.

The halophyte *Tripolium pannonicum* is able to uptake the antimicrobial sulfadimidine (SDI) and its biomass containing SDI produces high amount of methane through the anaerobic digestion process in comparison to other energy crops. In addition, biodegradation of SDI is carried out in the plant tissues and during anaerobic digestion.

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Acknowledgments

I would like to thank **Prof. Dr. Jutta Papenbrock** for inspiring and supporting me during my doctoral studies, for not only being an excellent supervisor, but also for her sincere and warm friendship. My words alone are insufficient to describe all her help and I will forever be grateful.

I would like to thank **PD Dr.-Ing. Dirk Weichgrebe** for taking over the function as second supervisor and teaching me many aspects about biogas production. I am also very grateful to **Prof. Dr. Thomas Dockhorn** for agreeing to be the external examiner, as well as **Prof. Dr. Marcus Andreas Horn** for being the chairperson of the doctoral committee.

Furthermore, I would like to thank **Pamela von Trzebiatowski** and **Julia Volker** for their general help in the laboratory, and **Yvonne Leye** and **Lutz Kruger** for their friendship and taking care of the plants.

I wish to acknowledge the financial support of my PhD by the Deutsche Akademische Austauschdienst –**DAAD**- and the Universidad de San Carlos de Guatemala –**USAC**- (Personal identification number: 91548278 funding programme: ALEGUA (57049520)).

I would also like to thank all members of the **AG Papenbrock** for being a true work team and creating a pleasant work environment.

Finally, I would like to thank my wife **Lilian Méndez de Turcios** for her patience, love and support during all this time as well as my **family** and **friends** for their support.

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01.2006-12.2007	Fakultät für Agrarwissenschaftlichen und Fakultät für Chemie und Pharmazie, Universität San Carlos von Guatemala –USAC-, Guatemala Stadt
	Mitarbeiter am Lehrstuhl für Mathematik und Physik

PRAKTIKA

01.2008-11.2008 Escuela Nacional Central de Agricultura -ENCA- (Landwirtschaftsschule), Guatemala

- Assistenzlehrer der Mathematik, Anorganische Chemie, Organische Chemie, Allgemeine Biologie und Fruchtbarkeit der Böden
- Assistenzlehrer des Moduls "Produktion von Gemüse in Gewächshäusern".
- Assistenzlehrer des Moduls "Getreideerzeugung".
- Assistenzlehrer des Moduls "Agrarforschung".
- Berater-Supervisor-Assistenzlehrer der Schüler in der landwirtschaftlichen und Forstwirtschaft Praxis (PAFS), in Zuckerrohrpflanzen

SPRACHKENNTNISSE

In Übereinstimmung mit dem Gemeinsamer Europäischer Referenzrahmen für Sprachen:
Spanisch – Muttersprache
Deutsch – B2 Niveau
Englisch – C1 Niveau

RELEVANTE AUSZEICHNUNGEN

Preis der Christian-Kuhlemann-Stiftung für die hervorragende wissenschaftliche Leistung und das gesellschaftliche und interkulturelle Engagement. Leibniz Universität Hannover, Dezember 2015

Auszeichnung für die beste Masterarbeit, Fakultät für Ingenieurwissenschaften, USAC, Guatemala Stadt, Juni 2012

Erster Platz bei der Top 10 der besten Studenten während des Bachelor-Studiums der Fakultät für Agrarwissenschaften der Universität USAC, Guatemala Stadt, Juni 2007

RELEVANTE KONFERENZVORTRÄGE

Biogas and methane production using biomass of the halophyte *Salicornia* spp. 14th World Congress of Anaerobic Digestion, Viña del Mar, Chile, November 2015

Elementanalysen von salzhaltigem Pflanzenmaterial mittels ICP-OES als Substrat in einem Fermentationsprozess. Anwendertreffen ICP-OES, Thermo Scientific. Veranstaltungsort: Institut für Anorganische Chemie, Leibniz Universität Hannover, November 2015

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Turcios A.E., Weichgrebe D., Papenbrock J (2015): Biogas and methane production using biomass of the halophyte *Salicornia* spp. Book of Abstracts from 14th World Congress of Anaerobic Digestion, Viña del Mar, Chile, 2015

Turcios AE, Weichgrebe D, Papenbrock J (2016a): Effect of salt and sodium concentration on the anaerobic methanisation of the halophyte *Tripolium pannonicum*, Biomass & Bioenergy. 87: 69-77. doi: 10.1016/j.biombioe.2016.01.013

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Cejas I, Rumlow A, Turcios AE, Engelmann F, Martínez ME, Yabor L, Papenbrock J, Lorenzo JC (2016): Exposure of Common Bean Seeds to Liquid Nitrogen Modifies Mineral Composition of Young Plantlet Leaves, American Journal of Plant Sciences, 7 (12): 1612-1617. doi: 10.4236/ajps.2016.712152