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FERMENTATIVE PROCESSES FOR ENVIRONMENTAL REMEDIATION

PhD student

Selene Grilli

PhD Coordinator

Prof. A. HOCHKOEPPLER

PhD supervisors

Prof. A. HOCHKOEPPLER
Prof. F. FAVA
Dr. A. SPAGNI

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Chapter 1

GENERAL INTRODUCTION

1.1. Environmental biotechnology and biorefinery

"*Sustainable development should become the basis for the life of future generations as opposed to over-exploitation of non–renewable energy and material resources and the shortening of life cycles*." (Conde et al., 2012).

The uncontrolled use of fossil fuels and finite natural resources, as a consequence of continuous urbanisation, industrialisation and mismanagement of renewable resources, has played key roles in climate change and degradation of various global ecosystems (Conde et al., 2012). Accordingly in recent decades, the growing interest in environmental protection has led to the development of remediation strategies for environmental issues introducing the biotechnology concept. Biotechnologies, indeed, could greatly support the change from the overexploitation of non-renewable resources to reach the goal of sustainability (OECD, 2004; Zechendorf, 1999).

Biotechnology is defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (Convention on Biological Diversity). Biotechnologies refer to four main domains: agricultural biotechnologies (GREEN), industrial biotechnologies and biotechnologies for environmental remediation (WHITE), biotechnologies in aquaculture (BLUE) and biotechnologies for medical applications (RED) (Black et al., 2011).

The international consultancy Frost & Sullivan (2010) defines Mega Trends as global, sustained and macroeconomic forces of development of our future. The research within the White Biotechnology framework reports the need by 2020 to implement new technological solutions for the treatment and management of the water cycle and waste both in municipal and industrial field.

In this context, the definition of environmental biotechnology implies a synergistic interaction of scientific and engineering knowledge that uses microbial agents and their products in strategies of remediation for the real and potential risks of environmental pollution (Conde et al., 2012; Wang L.K., 2010).

Since environmental concerns are directing towards the application of biotechnology for pollution control and waste minimisation, as well as, for the production of environmentally friendly products (bio-chemicals, See Chapter 10), the recent biotechnologies need to be applied in several anthropogenic sectors such as industry, agriculture, household, health, environment and others (Gavrilescu, 2010).

Due to the rising cost and environmental impact of fossil fuels, the search for new white biotechnologies has gained significant achievement in recent years. In this context, the biorefinery concept has been proposed. Biorefinery is a combination of processes and technologies to increase the economic viability for the future production of a wide range of value-added products, including biofuels and specialty chemicals (Conde et al., 2012; Black et al., 2011; Clomburg and Gonzalez, 2013). Unlike conventional refinery, biorefinery produces biofuels through the conversion of biomass as the main feedstock, such as agricultural residues and edible and non-edible crops (Zechendorf, 1999). Although the most commonly used feedstocks can reach high efficiency of conversion on an industrial scale, they are expensive and non-sustainable due to various commercial, economic and political factors (Clomburg and Gonzalez, 2013).

The environmental and economic benefit of the use of modern biotechniques has impacted various remediation technologies involved in the research of new disciplines (i.e. biochemistry, molecular and cellular biology, environmental engineering and others) concerning with the production of low toxicity products, new sustainable materials and renewable fuels from biomass and organic wastes.

On the other hand, environmental biotechnology is not a new area of interest, since some of the topics of concern are the current biotechnologies such as, fermentations, conventional wastewater treatments, composting, etc, (Clomburg and Gonzalez, 2013; Gavrilescu M., 2010).

Therefore, due to the environmental and economic concerns about fossil fuels, research is trying to develope industrial biotechnologies with the aim of enhancing the biodegradation, detoxification and minimisation of environmental pollutants in municipal and industrial wastewater and solid waste, as well as to improve bioenergy production in

order to reduce the use of fossil-fuels (Clomburg and Gonzalez, 2013; Gavrilescu, 2010; Wang et al., 2010).

1.2. Environmental biotechnologies

The removal of pollutants from water and waste treatment can be performed by physical, chemical, physicochemical, or biological (biotechnological) methods.

The advantages of the biotechnologies include the applicability of different process conditions by microbial agents for the treatment of a wide range of pollutants. Moreover, contrary to physico- and chemical methods which use expensive reagents, they are considered cheaper and environmental friendly.

The disadvantages of the biological methods are the requirement of nutrients and electron acceptors to maintain optimal conditions in the treatment system, and the relative process instability of conventional reactors due to unexpected behaviour of the involved microorganisms.

The biological processes for the wastewater and waste treatment mainly consist in aerobic, anaerobic and combination of aerobic and anaerobic processes (Wang et al., 2010).

1.2.1. Aerobic biological treatment

Aerobic microorganisms require oxygen as a terminal acceptor of electrons from organic or inorganic substances. The transfer of electrons from donor to acceptor is a source of biologically available energy.

The basic microbial reactions during aerobic biotreatment can be outlined as follows:

$$Organic\ material + O_2 \rightarrow CO_2 + H_2O + new\ cells \quad (1)$$

Cell mass of the new microorganism is gradually auto-oxidised in the decay processes:

$$Cells + O_2 \rightarrow CO_2 + H_2O + NH_3 \quad (2)$$

Wastewater

The source and the characteristics of the liquid waste largely influence the use of microorganism for the wastewater biotreatment. Wastewater can be mainly generated from municipal, industrial and agricultural sources and the aerobic processes are successfully used to treat municipal and industrial effluents. The microorganisms in the aerobic treatment plant can be exploited as suspended (activated sludge; see Chapters 3 and 4) or attached growth (fixed film) (Gavrilescu, 2010; Wang et al., 2010); (see Chapters 3 and 4).

The optimisation of the biological treatment can be obtained by the application of pre-treatments that mainly include mechanical disintegration-suspension of the particles, physical, and chemical separation and oxidation processes (see Chapter 6). It is noteworthy, that the xenobiotics (such as azo-dyes, see Chapters 3 and 4) can be effectively biodegraded by aerobic bacteria (Wang et al., 2010).

Solid waste

Biological (or organic) waste generated from various anthropogenic activities can be briefly classified as manure, sewage sludge as well as industrial and municipal wastes.

The unstable (highly fermentable) organic fraction of solid waste can be aerobically converted into a more stabilised product, later used as an organic fertiliser or disposed of (Gavrilescu, 2010).

The resulted advantages from the solid waste biotreatment (see Chapters 7 and 8) are reduced volume, stabilised material and low potential risk for the environment and human health by destructing of pathogens.

The biotreatment of solid waste under monitored aerobic condition is largely performed by s*oil bioremediation* strategies used in- or on-sites of post-accidental wastes; *composting*, as the widely applied bioremediation methodology, is used ex-situ (Gavrilescu, 2010; Wang et al., 2010).

1.2.2. Anaerobic biological treatment

The anaerobic biological process performs the microbial degradation and stabilisation of organic matters in absence of oxygen, and leads to the generation of biogas (a mixture of carbon dioxide and methane mainly) and biomass formation. The anaerobic process primarily consists in three sequential steps: hydrolysis (of carbohydrates and proteins),

fermentation and methanogenesis performed by several mixed bacteria species. The latter are anaerobic (living without oxygen), facultative anaerobic (living under anaerobic or aerobic conditions) (see Chapter, 10) and micro-aerophilic (preferring to live under low concentrations of dissolved oxygen) microorganisms (Wang et al., 2010).

The anaerobic microorganisms called tolerant anaerobes have protection mechanisms against oxygen, while obligate anaerobes cannot survive under aerobic conditions.

Obligate anaerobes produce energy from: a) fermentation (degradation of organic matter without external electron acceptors); b) anaerobic respiration using inorganic electron acceptors, such as CO_2, NO^-_3, NO^-_2, Fe_3^+,SO_4^{2-}; c) anoxygenic ($H_2S \rightarrow S$) or oxygenic ($H_2O \rightarrow O_2$) photosynthesis.

The energy yield (per mole of transferred electrons) of anaerobic respiration is usually much higher than fermentation (Wang et al., 2010).

Anaerobic processes are characterised by low capital costs (since they do not require oxygen/air addition) but have slower kinetics than aerobic ones; moreover, during fermentation or anaerobic respiration significant amount of dissolved organic products can be released (Wang et al., 2010).

Wastewater

Anaerobic biotreatment of wastewater does not typically result in low pollution levels so it is often considered as a pre-treatment process (Gavrilescu, 2010); (See Chapters 3, 4 and 5).

On the other hand, effluents containing high organic loads (e.g., cheese whey; see Chapter 9) should be treated by anaerobic process due to the possibility to obtain energy recovery as biogas and low quantity of biological excess sludge by means high efficiency treatment. Therefore, domestic and industrial wastewater can be considered a cost-effective potential source of energy (methane) from local feedstock (Chatzipaschali and Stamatis, 2012; Peixoto et al., 2012).

Different biotechnologies perform the anaerobic wastewater biotreatment by means suspended microorganisms, biofiltration (see Chapters 3, 4 and 5) and upflow anaerobic sludge blanket reactors (Wang et al., 2010).

Solid waste

The large variety of solid wastes is mainly generated by domestic, industrial and agricultural activities.

The most common anaerobic biotreatments of the solid waste include anaerobic digestion (for the biological stabilisation) and the codigestion (cofermentation) with effluents from various sources as municipal, zootechnical, agricultural and industrial. Landfilled waste is slowly biodegraded by anaerobic microorganisms.

Over the last decade, there has been a growing interest on anaerobic digestion since methane can be produced as end-product.

1.2.3. Combining aerobic and anaerobic biotreatment

A combined anaerobic-aerobic biotechnology can reach higher efficiencies of treatment than aerobic or anaerobic treatment alone.

This treatment consists of a combination or alternation of anaerobic/anoxic and aerobic processes able to enhance the biodegradation by increasing the removal efficiencies of carbon, nitrogen and phosphorus (Wang et al., 2010).

Therefore, the monitoring of aeration conditions is the major parameter for maintaining the stability and maintenance costs in this typology of biological technology.

1.3. Fermentative processes

Anaerobic processes are usually preferred for waste and wastewater treatment in comparison with other physicochemical and biological methods. Anaerobic fermentative processes, compared to conventional aerobic processes, include a series of advantages like, for instance, lower energy consumption, lower production of excess sludge, higher energy efficiency, simpler process, more suitability for high organic loads, and, above all, it produces biogas containing methane as final gaseous product for energy use (Chatzipaschali and Stamatis, 2012; Gavrilescu, 2010).

The fermentation is a promising cost effective, commercially viable and sustainable process to be applied in the biotechnological treatment of organic waste and wastewater. Unlike many other treatment technologies, it is also capable of simultaneous valorisation of specific feedstocks and energy recovery, in relatively short time (Clomburg and Gonzalez, 2013; De Meester et al., 2012; De Peixoto et al., 2012).

In the framework of the recent remediation strategies, the use of White Biotechnologies has favoured the application of fermentative process to convert the renewable substrates, largely available, to chemicals and energy (De Meester et al., 2012; De Peixoto et al., 2012; Soetaert and Vandamme, 2010).

In this context, the present thesis has been focused on the investigation of fermentative biotechnologies that were implemented for environmental remediation and bioenergy production. The research activity dealt with four different applications as better described in the following paragraph.

1.4. Structure of the thesis

This thesis is organised according to the following structure.

In this chapter (Chapter 1), a general introduction and the rational of the study is presented.

The following short chapter (Chapter 2) describes in detail the aims of the study.

Then the thesis is organised according to the experimental studies that have been carried out. These experimental studies were structured in four different lines that were dealt during the Doctorate. The lines of research cover the broad subject of biorefinery concept.

The four research lines were:

1. Biotechnology for textile wastewater treatment and water reuse;
2. Biotechnology for solid waste treatment and management;
3. Bioenergy production from agro-zootechnical waste;
4. Fine chemicals production from waste of the bioenergy production industry.

Research line 1 was examined in chapters 3, 4, 5 and 6 and it concerned the treatment of effluents from the textile industry.

Biotechnologies involving anaerobic and aerobic processes were investigated in combination with membrane technologies to obtain treated water suitable for the reuse inside the textile company. In particular, while Chapters 3 to 5 investigate biotechnological processes, Chapter 6 presents an investigation on the application of different strategies/scenarios, including biotechnological treatment of wastewater, for water reuse in the textile manufacturing processes.

Research line 2, reported in Chapters 7 and 8, dealt with the biological treatment of the solid waste and leachate in landfill.

Chapter 7 describes the possible fate of the residue after the bio-drying process for the stabilisation of the municipal solid waste organic fraction if disposed of in landfills.
Landfill operated as Bioreactor with recirculation of the generated leachate was proposed as novel biotechnology for solid waste organic fraction stabilisation and leachate treatment in combination with energy (biogas) production. Chapter 8 evaluates the nitrogen removal processes during leachate recirculation in the landfill.
The **research line 3** is described in Chapters 9 and it concerned the codigestion of the solid and liquid wastes from the agro-zootechnical dairy industry. An innovative biotechnology with a simplified design was studied to enhance the treatment efficiency over the conventional processes ones.
The **research line 4** is reported in chapter 10 and it took into account the valorisation of the waste glycerol as the main by-product of the biodiesel industry.
Since, the considerable increase in biodiesel production has resulted in excess coproduction of crude glycerol, this study evaluated the feasibility of the microbial conversion of this substrate to value-added chemicals as 1,3-propanediol. A biotechnological fermentative process able to convert crude glycerol without any pre-treatment was investigated in order to improve the competitiveness of the biodiesel industry.
Finally, Chapter 11 reports the main conclusions of the thesis with a general evaluation of the proposed biotechnologies for environmental remediation.
This experimental activity was carried out at the ENEA Water Resource Management Laboratory (Bologna) and at the Department of Civil, Environmental and Materials Engineering (DICAM) of Bologna University Engineering Faculty. In particular, the research lines 1 and 2 were carried out in the ENEA's laboratories, while the latter two research lines were realised in the laboratories of the University of Bologna.

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Chapter 2

AIMS

This thesis evaluated the applicability of biotechnological applications for the environmental remediation and bioenergy production. In particular the study aimed at evaluating the valorisation of waste generated in different industrial sectors, such as textile, dairy or biodiesel production.

The suitability of emerging white biotechnologies for solving specific environmental issues concerning the four research lines (see Chapter 1) of this thesis were investigated, in accordance with the biorefinery concept.

The optimisation of processes configuration under anaerobic and aerobic conditions in membrane bioreactors and suspended- and attached-growth biosystems was performed.

Innovative designs were also applied for enhancing performance and reducing footprint of the investigated technologies.

Moreover, the possible implementation of the proposed biotechnology was also evaluated in a case study applied to a textile company (see Chapter 6).

The research activity was mainly related to the application of fermentative processes that were applied alone or in combination with other technologies.

The specific objectives of the research activities as delineated in the four research lines (see Chapter 1) are described below.

The **research line 1** aimed to evaluate the application of different biotechnologies for the treatment of textile wastewater.

Diverse process configurations combining anaerobic and aerobic biological treatments with membrane technologies were examined. The purposes of the treatments were the optimisation of the colour removal and making the processes economically feasible to the treatability of the effluents and the reusability of the treated water in the textile processes.

An additional focus was on the proposal of a methodology designed on a different combination of physical, chemical and biological treatments to be applied to highly variable discharges from various industrial sectors.

The specific objectives of **research line 2** were related to the anaerobic management of municipal solid waste. The study aimed to evaluate the effect of the addition of the fine fraction, resulted from the bio-drying process of the organic fraction of municipal solid waste, to a landfill operated as bioreactor. The stabilisation of the waste as biogas production potential was evaluated.
Moreover, this research line also aimed to evaluate the fate of ammonia when a bioreactor is managed as bioreactor. Therefore, a pilot-scale landfill bioreactor was investigated with the aim of performing the Anammox process for the anaerobic nitrogen removal.

The **research line 3** was concerned with the energetic valorisation of wastes from dairy industry, such as cheese whey and cattle manure.
Different process configurations were investigated with the aim to identify the suitable fermentative processes for the optimal codigestion of the two substrates. A novel biotechnology with simple design and reduced footprint was implemented in order to combine the maximum efficiency both of codigestion and methanisation.

The **last research line** aimed to the valorisation of the crude glycerol resulted as the main by-product of the biodiesel manufacturing processes.
The conversion of glycerol waste to value-added chemicals, such as 1,3-propanediol, by microbial fermentation was investigated with the objective to maximise the yield of the target product. The inhibition on microbial metabolism due to the substrate and the by-products of the fermentative process, above all when present in high content, was also investigated.
The potential of using glycerol waste at industrial level without any pre-treatment was the key focus of the proposed fermentative processes in order to implement a sustainable and economic fermentative biotechnology.

Chapter 3

TREATMENT OF A SIMULATED TEXTILE WASTEWATER CONTAINING THE AZO-DYE REACTIVE ORANGE 16 IN AN ANAEROBIC-BIOFILM ANOXIC-AEROBIC MEMBRANE BIOREACTOR*

Abstract

This study evaluated the treatability of simulated textile wastewaters in a bench-scale experimental system, comprising an anaerobic biofilter, an anoxic reactor and an aerobic membrane bioreactor. The Reactive Orange 16 (RO16) was used as model of azo dye. The proposed system was demonstrated to be effective in the treatment of the synthetic wastewater under the operating conditions applied in the study. The results demonstrate that neither the azo dye, nor the aromatic amines formed by the anaerobic azo-bond cleavage seem to significantly affect the COD and nitrogen removal under the operating conditions applied. Although aromatic amines are considered easily degradable under anaerobic conditions, the results confirms that at least the sulfonated aromatic amines formed under anaerobic conditions from the RO16 are recalcitrant to biodegradation and therefore aromatic amines are still a matter of concern for the biological treatment of textile wastewater.

Keywords: Biofilm, Membrane bioreactor, Textile wastewater, Azo-dye, Aromatic amines.

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3.1. Introduction

Dyes are used in different industrial sectors, among which the textile industries is one of the most significant users. The manufacture of several textile products involves the use of numerous different dyes and auxiliary chemicals (e.g. salts) in many different industrial processes that cause the formation of wastewaters with complex and very variable characteristics that makes their treatment particularly difficult. The textile industry is also one of the most water-consuming industrial sectors (Correia et al., 1994; Delee et al., 1998; O'Neill et al., 1999; Vandevivere et al., 1998).

Among the different classes of colorants, azo dyes are the most used (O'Neill et al., 1999). Azo dyes are characterised by one or more nitrogen-nitrogen double bond (-N=N-) called azo group. Their removal from wastewater can be accomplished by physical, chemical or biological processes, but biological processes are usually preferred because they are cheaper and environmentally friendly.

Environmental conditions or process operations greatly influence azo dye removal and textile wastewater treatment (Carliell et al., 1995; Pandey et al., 2007; Rai et al., 2005; Stolz, 2001). The azo bound is stable to aerobic biodegradation, whereas it is quite easily reduced under anaerobic biotreatment with the elimination of colour but with the formation of potentially harmful aromatic amines. Both biological and chemical reactions take place under anaerobic biotreatment of azo dyes (dos Santos et al., 2004), although the biological processes seem to be more important for colour removal (van der Zee et al., 2003). Contrary to the azo group, aromatic amines are, with a few exceptions (Razo-Flores et al., 1997), stable under anaerobic conditions whereas are aerobically biodegradable. Therefore, biological treatment of wastewaters containing azo dyes (e.g. textiles) is usually accomplished with the combination of anaerobic and aerobic conditions (Dos Santos et al., 2007; Pandey et al., 2007; Rai et al., 2005; Vandevivere et al., 1998; van der Zee and Villaverde, 2005).

Although over the last decades several studies on azo dyes biodegradation have been reported, in most of them (even recently published), only decolourization was described whereas no data on the fate of the formed aromatic amines were provided. Moreover, even though aromatic amines have been considered easily biodegradable under aerobic conditions, some researchers have found evidence of the low biodegradability of the

sulfonated aromatic amines formed during the azo-bound cleavage of certain azo dyes (Lourenco et al., 2000; 2001; Tan et al., 1999; 2000).

Azo dye removal has been studied using both pure and mixed cultures (e.g. Lourenco et al., 2000; 2001; Tan et al., 1999; 2000). Although interesting results have been obtained using pure cultures (Elisangela et al., 2009; Ghodake et al., 2009; Parshetti et al., 2010), these seem to be not applicable at full scale for real textile wastewater treatment due to the presence of autochthonous microorganisms.

Membrane bioreactors (MBRs) consist of the combination of biological processes (typically the activated sludge process) with membrane technologies and are being applied when very high-quality effluents are required, for instance for water reuse (Meng et al., 2009; Van Nieuwenhuijzen et al., 2008). Over the last decades, membrane technologies alone or in combination with biological processes (i.e. MBRs) have been successfully applied for textile wastewater treatment (Mattioli et al., 2002; Vandevidere et al., 1998).

The aim of this study was to evaluate the applicability of an anaerobic/anoxic/aerobic system for the biological treatment of textile wastewaters. The proposed experimental system comprises an anaerobic biofilter for azo dye removal, an anoxic tank for COD and nitrogen removal, and an aerobic reactor for nitrification and amines removal. Because of the large complexity and variability of the textile wastewaters, this study pays attention not only to the fate of the dyes, but also to the nitrogen and COD removal. The treatment system comprised a microfiltration membrane operated as MBR, in order to evaluate the possibility of obtaining high-quality effluents suitable for water reuse. The Reactive Orange 16 (RO16) was used as model of azo dye. It should be highlighted that, due to the very high variability of textile wastewater (Correia et al., 1994; O'Neill et al., 1999; Vandevivere et al., 1998), this study addresses the treatability of the "low concentration" ones, as defined by Mattioli and Grilli (2010), in order to make the process economically feasible.

3.2. Materials and methods

Rector set-up

In this study, a bench scale reactor consisted of three compartments (an anaerobic biofilm, an anoxic tank and an aerobic MBR) was used (Fig. 1). The treatment system was maintained at 20 ±1°C in a thermostatic room.

The anaerobic biofilm has a total volume of 2.7 L; it was packed up to the volume of 1.4 L with Biomaster BCN 012 KL (Amitec, Italy) that was maintained fixed using a stainless steel net. The packing material has a cylindrical shape (12.5x12.5x12.5, LxWxH) with a cross separation inside and is made of polyethylene with density of 0.95g/cm^3, protected area of 500 m^2/m^3 and void degree of 86% (data from the supplier); a liquid volume of approximately 200 mL above the stainless steel net maintained the biofilm always completely submerged. The void volume of the biofilm resulted in 1.2 L.

The working volumes of the anoxic and aerobic tanks were 1.0 and 1.1 L, respectively. Internal recycle was carried out using a peristaltic pump (Watson Marlow 403U/R1) at a constant flow rate of 5.0 mL/min for the entire duration of the study. A second peristaltic pump (Watson Marlow 401U/D1), controlled by a level sensor in the reactor, was used for feeding.

A hollow fibre membrane module (ZW1, Zenon, Italy) with a membrane filtration area of 0.047 m^2 and nominal porosity of 0.04 μm (data from the supplier) was immersed into the aerobic tank.

Membrane filtration was carried out using a programmable piston pump (Ismatec, Cole-Parmer, USA) whereas aeration was obtained using an aquarium blower (flow at approx 80 L h^{-1}). To reduce fouling, the membrane was operated alternating cycles of 3 and 2 min of filtration and relaxation, respectively (no back wash). The transmembrane pressure was monitored using a digital gauge (Cole-Parmer, USA). Due to the low membrane fluxes applied, no severe fouling was observed during the experiments.

A mechanical stirrer (RZR, Heidolph, Italy) was used for mixing the anoxic tank.

A homemade wet gasmeter was used for biogas production monitoring.

Sludge withdrawal was performed manually in order to control the solid retention time at approximately 80-100 days.

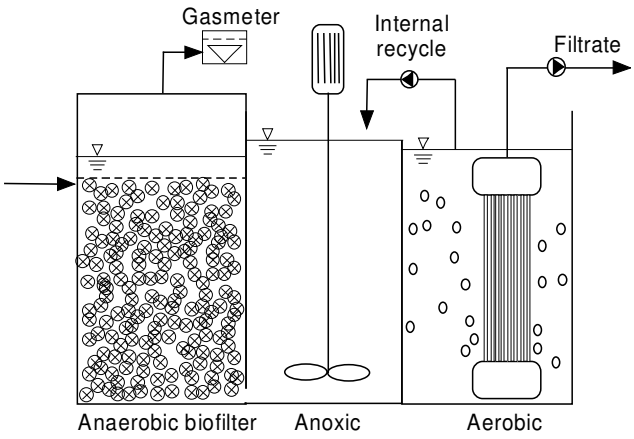

Figure 1. Schematic diagram of the bench-scale reactor.

Inoculum and synthetic wastewater

The Anoxic and the aerobic vessels were seeded with activated sludge taken from a full-scale municipal wastewater treatment plant. For the anaerobic vessel, before the beginning the study, the packing materials (used for supporting the biofilm) was left completely immersed for approximately two months in the anaerobic sludge (taken from the anaerobic sludge treatment of the same wastewater treatment plant) in order to promote the biofilm development. Then, at the beginning of the study, the packing material was transferred to the anaerobic vessel of the bench-scale plant; moreover, approximately 100 g of wet granular sludge taken from a full-scale upflow anaerobic sludge blanket treating agro-industrial wastewater, was added to the anaerobic vessel in order to improve the anaerobic processes of the bench-scale reactor during the start-up.

The bench-scale reactor was fed with synthetic wastewater composed of glucose as the main (the other possible carbon source was the azo dye) carbon source at a concentration of 800 mg COD L^{-1}, NH_4Cl (40 mg N L^{-1}), $NaHCO_3$ (240 mg L^{-1}) K_2HPO_4 (56.2 mg L^{-1}). Although tap water was used for the synthetic wastewater, the following salts were also added in order to assure sufficient presence of micronutrients (concentrations in mg L^{-1}): $CaCl_2$ (20), $MgCl_2*6H_2O$ (50), $FeCl_3$ (20), $ZnCl_2$ (5), $CoCl_2*6H_2O$ (5), $MnCl_2*4H_2O$ (5), $CuCl_2*2H_2O$ (5), $NaMoO_4*2H_2O$ (2), $NiCl_2*6H_2O$ (2), $AlCl_3$ (2), H_3BO_3 (0.5), Na_2SeO_3 (0.5). Moreover, because textile wastewater usually presents medium-high conductivity (Correia et al., 1994; O'Neill et al., 1999) NaCl was added (400-500 mg L^{-1}) to increase the conductivity of the synthetic wastewater to approximately 3 mS/cm.

During the study, the hydraulic retention time (HRT) was gradually decreased from 3.85 to 1 day (according to Fig. 2).

The reactive orange 16 (RO16; C.I. 17757) used in this study was kindly supplied by a textile factory. The compound has a dye content of 50% and was used without further purification or pretreatment. A variable influent RO16 concentration from 5 to 37.5 mg L^{-1} (10-75 mg L^{-1} of the azo dye compound) was applied.

Analysis

Chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS), ammonia, nitrite and nitrate nitrogen were measured according to the Standard Methods (APHA, AWWA, WEF, 2005). COD measurements of the effluents of the anaerobic biofilter and the anoxic vessel were performed on filtered samples. Filtration was carried out using Whatman GF/C filters.

Dissolved oxygen (DO) and pH were measured using Crison probes and instruments.

Glucose was measured using the phenol-sulphuric acid reaction (glucose as standard) (Dubois et al., 1956).

Azo dye concentration has been estimated by spectrophotometric measurement at its maximum absorption wavelength (484 nm).

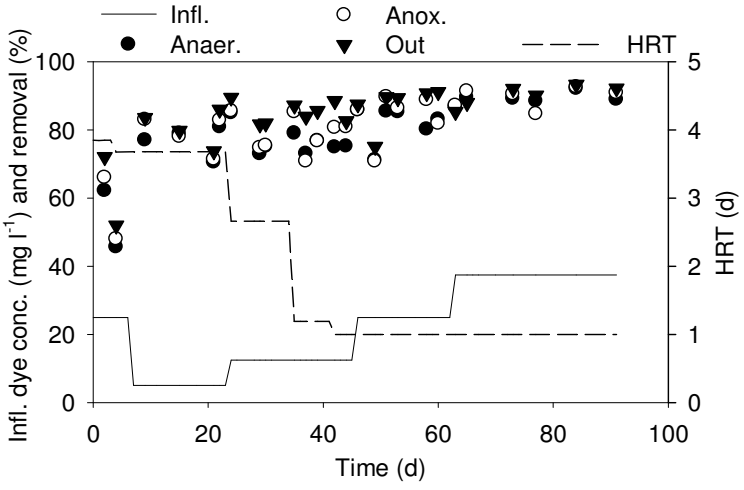

Figure 2. Hydraulic retention time (HRT), influent azo dye concentration, anaerobic, anaerobic and aerobic (out) colour removal.

An estimation of the total amines produced by the azo bond cleavage was obtained by the diazotization-coupling reaction with N-(1-naphthyl)ethylenediamine according to Norwitz and Keliher (1982); sulfanilic acid, which has a chemical structure similar to one of the two amines expected from the azo dye degradation, was used as standard for the calibration curve. Possible interference of the RO16 on amine measurement has been estimated being negligible (below 1%) in the experimental conditions of this study.
Volatile fatty acids (VFA) were determined by using a gas chromatograph (GC-Dani 8510) equipped with a capillary column (DB-FFAP, 30 m x 0.53 mm x 1.5 □m), a flame ionisation detector and using hydrogen as carrier gas. Biogas composition was measured by a second GC (Dani 3865) equipped with a column packed with Haye-sep"Q" (inner diameter 1 mm, length 2 m), a thermal conductivity detector and using nitrogen as carrier gas.

3.3. Results and discussion

Colour removal

The reactor was started up applying a low HRT (Fig. 2) in order to allow biomass acclimation to the operating conditions and to the synthetic wastewater.
At the azo dye concentration of 25 mg L^{-1} applied during start up, the effluent of the anaerobic biofilm as the effluent of the bench scale reactor, were coloured showing low efficiency in colour removal in anaerobic conditions. Therefore, the azo dye concentration in the influent was decreased in order to improve biomass acclimation. With the decrease of the influent azo dye concentration, a sudden improvement of the colour removal was observed (Fig. 3). The biomass acclimation to the azo dye and to the experimental conditions allowed the continuous improvement in colour removal although the influent azo dye increase and the HRT reduction (Fig. 2) which resulted in a relatively high azo dye loading rate. HRT was gradually decreased from 3.85 d until reaching the value of 1 d, a reasonable value for textile wastewater treatment (Delee et al., 1998; van der Zee and Villaverde, 2005), after 40 days of operation. Biomass
acclimation to the operating conditions is confirmed by the TSS increase (Fig. 3) in the aerobic tank. Colour removal mainly took place under anaerobic conditions, although a small increase in colour removal was still observed under anoxic and aerobic conditions.

Decolourisation increased from approx. 50% to 90% in 60 days. The small increase in colour removal under anoxic and aerobic conditions can be attributed to two main factors. Firstly, a reducing activity, manly under anoxic conditions, where small anaerobic microzones (e.g. inside the activated sludge flocks) can be present; secondly, a microbial activity which remove other organic compounds (present in the influent or produced during biomass decay) with light absorbance in the monitored wavelength (484), can overestimate the azo dye colour removal.

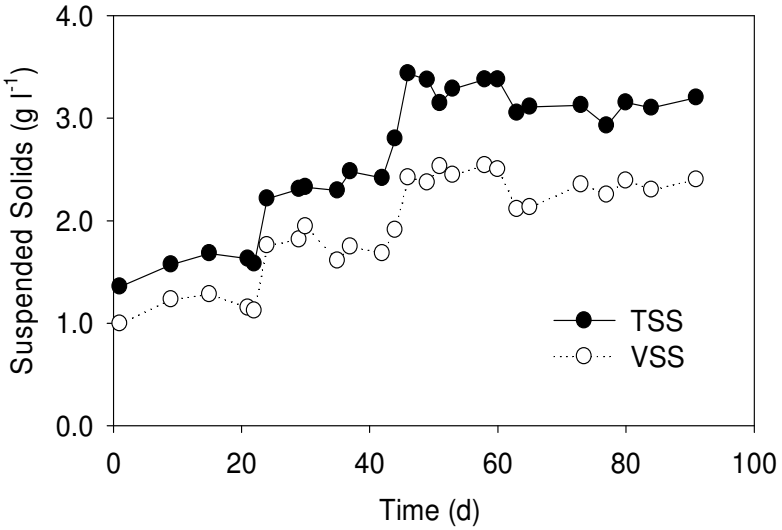

Figure 3. TSS and VSS concentrations in the aerobic tank.

The anaerobic treatment caused the formation of aromatic amines (Fig. 4). Although the presence of anoxic and aerobic processes combined with the microfiltration/ultrafiltration unit (MBR), the formed amines were not removed in the treatment system: ammines concentration increased according to the increased azo dye loading. The results, thus, confirm that the aromatic amines can be recalcitrant under aerobic biodegradation (Pandey et al., 2007). Therefore, the possible formation of aromatic amines during the biological treatment of textile wastewaters containing azo dyes, remains a matter of concern. Over the last decade some studies have evidenced the difficulties in degrading sulfonated aromatic amines, the amines presumably produced under anaerobic conditions from RO16 (assumed from the chemical structure of the azo dye). For instance, Lourenco et al. (2001), alternating anaerobic and aerobic conditions in a sequencing batch reactor, obtained significant azo dyes removal (Remazol Brilliant Violet 5R and Remazol Back B),

but with no improvement in the aerobic removal of the aromatic amines produced under anaerobic conditions. Moreover, in a very extensive study on sulfonated aromatic amines, (Tan et al., 2005) highlighted that of ten tested sulfonated aromatic amines only two were degraded in aerobic conditions and an extensive biomass acclimation was necessary for the biodegradation. Among the ten aromatic amines tested, sulfanilic acid (p-aminobenzene-sulfonic acid), an aromatic amine with chemical structure similar to the one expected in this study, was found biodegradable using inocula very well acclimated to the pollutant (Tan et al., 1999; 2005). On the contrary, other studies showed that sulfanilic acid was not degraded by municipal activated sludge (Tan et al., 1999; Yemashova and Kalyuzhnyi, 2006). In a recent study, Carvalho et al. (2008) showed that sulfanilic acid can also be removed by conventional activated sludge (municipal or industrial), but a quite long lag phase was observed (approx. 100-200 h).

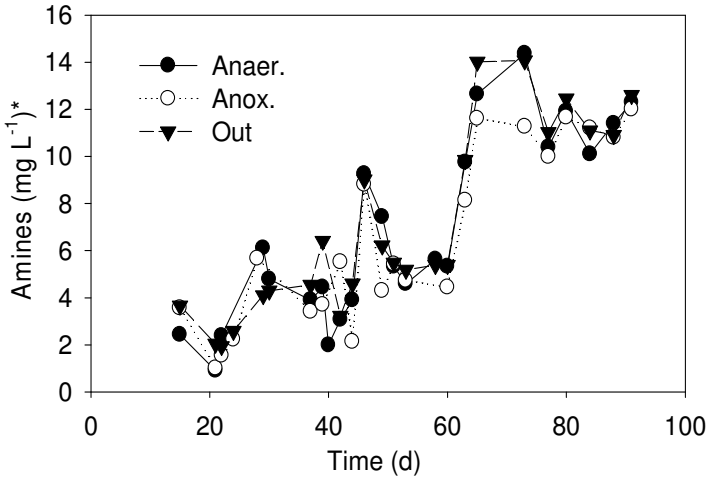

Figure 4. Amine concentration in the effluent of the anaerobic biofilter, in the anoxic and the effluent of the reactor. * Concentration as sulfanilic acid (see materials and methods).

COD removal

The reactor was fed with synthetic wastewater at a constant COD concentration of 800 mg L^{-1}. The increased loading rate (due to the decreased HRT according to Fig. 2) resulted in an increased effluent COD concentration from the anaerobic biofilm (Fig. 5a). Anaerobic COD removal decreased from the maximum value of 79% measured on day 7 to 40 % at the end of the study. Although the increased effluent COD of the anaerobic biofilter, the

COD removal efficiency increased almost continuously up to 350-450 mgCOD L^{-1} d^{-1} in approx. 35 days, afterward stabilising at a slightly lower values of approx. 300-350 mgCOD L^{-1} d^{-1} indicating that the maximum anaerobic COD removal was achieved (Fig. 5b). The maximum anaerobic COD removal is confirmed by the presence of glucose in the effluent of the anaerobic biofilter when the HRT was decreased below 3 d (Fig. 6a). The increased loading rate applied to the anaerobic biofilter with the biomass acclimation to the operating conditions and to the azo dye, caused the increase in biogas production (Fig. 6b) from values close to zero to approx. 200-600 mL d^{-1} (methane content approx. 55-65%). Biogas yield seems to be not greatly influenced by the azo dye presence in the feed due to its relative very low amount compared to the total COD, as also observed by Carvalho et al. (2008).

Due to the relatively low organic loading applied to the anaerobic biofilm compared to the typical anaerobic digestion processes, the VFAs were produced at very low concentrations: among the VFAs monitored, acetic acid presented the highest concentration but was always below 70 mg L^{-1}, whereas most of the other VFAs (especially those with higher molecular weight) were below the detection limit of the instrument (10-20 mg L^{-1} depending on the VFA; data not showed).

As for the biogas production and glucose concentration, other process parameters showed high variability (e.g. pH, data not shown): this high variability might be caused by wastewater short-circuiting in the biofilter due to the very small volume of the reactor. The anaerobic COD removal caused a decrease of the organic load applied to the anoxic-aerobic MBR which in turn reduced the activated sludge biomass growth. In fact, the solids concentrations stabilised at relatively low values of about 3.1-3.2 and 2.3-2.4 g L^{-1} for TSS and VSS, respectively, although the high SRT (80-100 d). Due to the presence of the microfiltration unit, solids in the effluent were absent.

Therefore, the presence of the azo dye at the concentration applied in this study seems not to considerably influence the anaerobic processes at least after biomass acclimation.

The effect of the increased load was also observed on the anoxic and the effluent COD, although with a much less extent (Fig. 6a); the COD concentrations increased from approx. 80-100 to 150-200 mg L^{-1} in the anoxic tank and from 40-50 to 80-100 mg L^{-1} in the effluent. The results of the MBR on COD removal confirmed the high stability of the combination of the biological processes with the membrane filtration (Meng et al., 2009; Van Nieuwenhuijzen et al., 2008).

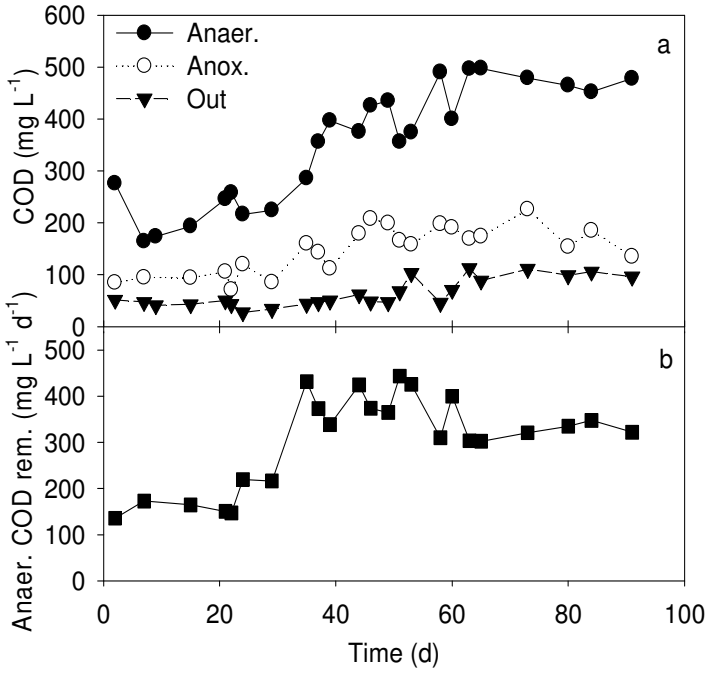

Figure 5. COD concentration in the effluent of the anaerobic biofilter, anoxic tank and the treatment system (a); COD removal efficiency in the anaerobic biofilter (b).

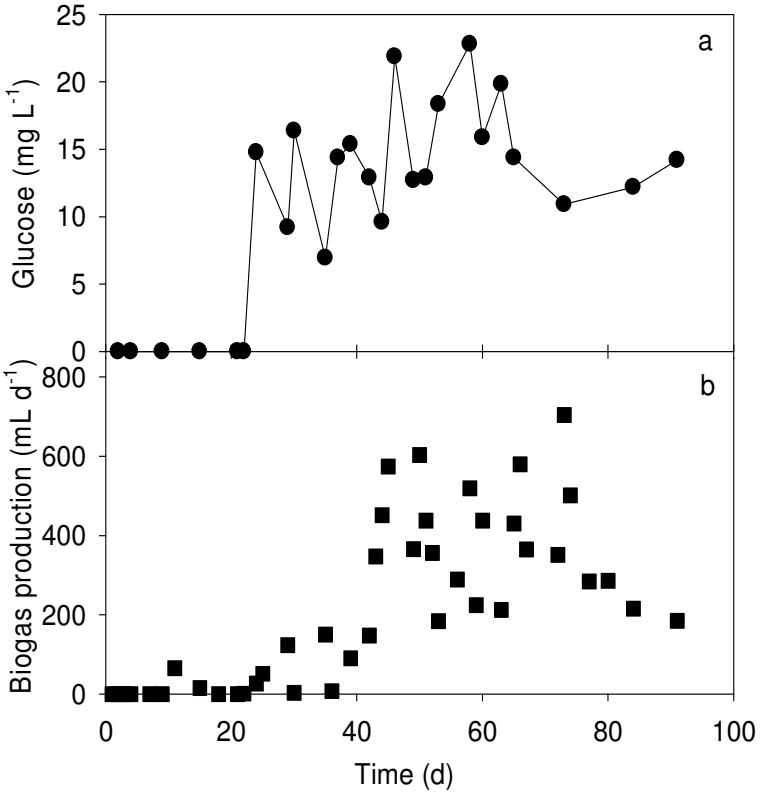

Figure 6. Glucose concentration (a) and biogas production in the anaerobic biofilter (b).

Nitrogen removal

Because textile wastewaters are often treated in conventional activated sludge, this study also evaluated possible interference/inhibition on the nitrification processes.

The system showed always good ammonia removal (Fig. 7); in fact, the effluent ammonia concentration was always below 7.0 mgN L^{-1} (mean 1.5; SD 1.4 mgN L^{-1}; Fig. 7a). On the contrary, nitrite and nitrate removal were related to COD availability (Fig. 7b and 7c). In fact, the nitrogen removal increased and stabilised when the organic loading to the reactor was increased reducing the HRT. The increased organic loading increased the effluent COD of the anaerobic biofilter (see section 3.2.) leaving sufficient organics for the denitrification processes (Fig. 7c). Small effluent nitrite peaks coinciding with the HRT decrease were observed during the study indicating that the azo dye and/or the produced amines could slightly inhibit the nitrification process during the applied operating conditions. However, biomass acclimation also seems to improve the nitrification process.

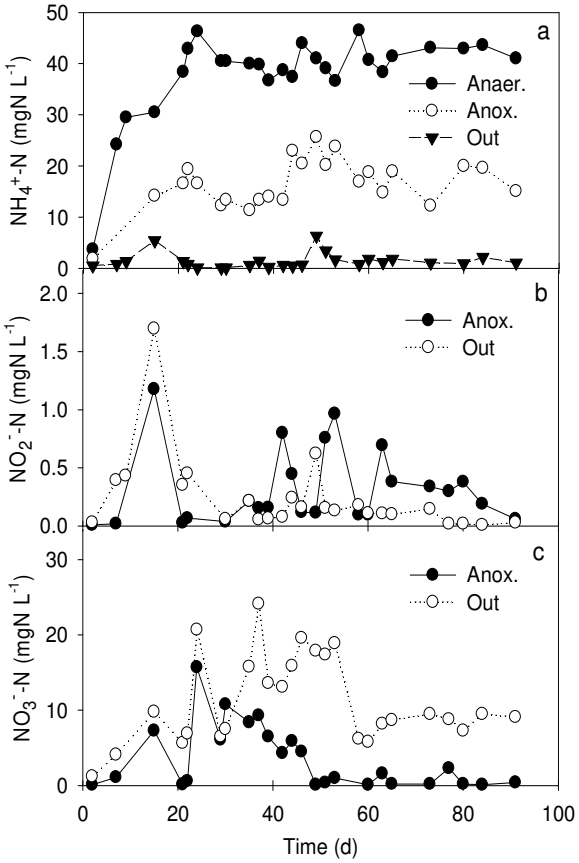

Figure 7. Ammonia (a), nitrite (b) and nitrate (c) concentration in the anaerobic biofilter, anoxic tank and effluent of the treatment system.

The reduced nitrate removal observed under anoxic conditions during the first 40 days of experimentation might also be due to the high DO concentrations of the aerobic tank (always above 7.0 mgO_2 L^{-1}) which might have caused a significant oxygen flow from the aerobic to the anoxic reactor through the internal recycle (Fig. 1). The high DO concentration was caused by the high aeration rate required for membrane scouring in order to reduce membrane fouling. Moreover, the internal recycle having been maintained fixed (see section 2.1.), the effect of the internal recycle with high HRT (i.e. during the first 40 d) was relatively greater.

The high aeration rate in the aerobic vessel also affected pH. The pH values were 7.31 (SD 0.12), 7.18 (SD 0.22), 7.60 (SD 0.32), 8.24 (SD 0.21) in the influent, anaerobic, anoxic and aerobic reactor, respectively. It is well-known that under anaerobic conditions pH of the bulk liquid tends to decrease due to fermentative processes. On the contrary, under anoxic conditions pH tends to increase due to alkalinity production caused by the denitrification processes, whereas, nitrification processes have an acidifying effect (Spagni et al., 2007). In this study, contrary to what was expected, pH showed the highest values under aerobic condition (data not shown). The high aeration rate applied for membrane scouring, could have increased CO2 stripping, which thus has became prevalent on acidifying effect of the nitrification processes.

3.4. Conclusions

The results of the study demonstrate that a system comprising an anaerobic biofilter and an anoxic-aerobic MBR is suitable for synthetic textile wastewater treatment. Neither the azo dye, nor the aromatic amines formed by the anaerobic azo-bond cleavage seems to significantly affect the COD and nitrogen removal under the applied operating conditions. Although aromatic amines are considered easily degradable under anaerobic conditions, the results confirm that at least the sulfonated aromatic amines formed under anaerobic conditions from the RO16 are recalcitrant to biodegradation.

Acknowledgements

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Chapter 4

TEXTILE WASTEWATER TREATMENT IN A BENCH-SCALE ANAEROBIC-BIOFILM ANOXIC-AEROBIC MEMBRANE BIOREACTOR COMBINED WITH NANOFILTRATION*

Abstract

This study evaluated the treatability of textile wastewaters in a bench-scale experimental system, comprising an anaerobic biofilter, an anoxic reactor and an aerobic membrane bioreactor (MBR). The MBR effluent was thereafter treated by a nanofiltration (NF) membrane. The proposed system was demonstrated to be effective in the treatment of the textile wastewater under the operating conditions applied in the study. The MBR system achieved a good COD (90-95%) removal; due to the presence of the anaerobic biofilter, also effective colour removal was obtained (70%). The addition of the NF membrane allowed the further improvement in COD (50-80%), colour (70-90%) and salt removal (60-70% as conductivity). In particular the NF treatment allowed the almost complete removal of the residual colour and a reduction of the conductivity such as to achieve water quality suitable for reuse.

Keywords: Anaerobic Biofilm, Membrane Bioreactor, Nanofiltration, Textile Wastewater, Water Reuse.

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4.1. Introduction

The manufacture of several textile products involves the use of numerous different dyes and auxiliary chemicals (e.g. salts, organic compounds) in many different industrial processes (dyeing, bleaching, printing, washing, etc.) that produce wastewater with complex and very variable characteristics that makes their treatment particularly difficult. [1-4] The textile industry is also one of the most water-consuming industrial sectors: for instance, the textile dye process consumes even more than 100 L/kg of fabric processed. [5]

The textile industry uses several different classes of colorants (e.g. azo, anthraquinone, triarylmethane), among which the azo dyes are the most common. [4] Their removal from wastewater can be accomplished by physical, chemical or biological processes, but biological treatments are usually preferred because they are cheaper and environmentally friendly. [6-7] The biological treatment of textile wastewater is greatly influenced by process conditions. [8-11] For example, azo dyes are stable to aerobic biodegradation, whereas they are quite easily reduced under anaerobic biotreatment with the elimination of colour but with the formation of potentially harmful aromatic amines. Although for a few aromatic amines, characterised by hydroxyl and carboxyl substitutes, complete anaerobic mineralization has been observed [12] contrary to the azo bounds, aromatic amines are usually stable under anaerobic conditions whereas are aerobically biodegradable. Therefore, the biological treatment of wastewaters containing azo dyes (e.g. textiles) is usually accomplished with the combination of anaerobic and aerobic conditions. [3, 10-11, 13-14] Moreover, even though aromatic amines have been considered easily biodegradable under aerobic conditions, some studies have found evidence of the low biodegradability of the sulfonated aromatic amines. [15-18]

Over the last decades, membrane technologies have been successfully applied for textile wastewater treatment. [3,19] When membranes have been applied alone for textile wastewater treatment a train of filtrations has been implemented, usually including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and/or reverse osmosis. [20-21] Membrane bioreactors (MBRs) consist of the combination of biological processes (typically activated sludge) with membrane technologies and are being applied when very high-quality effluents are required, for instance for water reuse. [22-23] Moreover, MBRs have been considered as an ideal pretreatment when further filtration (nanofiltration or

reverse osmosis) have to be applied. [24] During the last decades NF has been extensively studied as the final treatment for water reuse in the textile factories and has been proposed as the elective technology (with reverse osmosis) for water reuse. [21,25-26]

The aim of this study was to evaluate the applicability of an anaerobic/anoxic/aerobic system, comprising an UF module, for the biological treatment of real textile wastewaters. In addition, NF was tested on the effluent of the MBR in order to achieve the water quality suitable for reuse in the textile industry.

4.2. Materials and methods

Reactor Set-Up

The proposed bench-scale experimental system consists of an anaerobic biofilter for (azo) dyestuffs removal, an anoxic tank for COD and nitrogen removal, and an aerobic reactor for nitrification and amines removal. The treatment system also comprises a micro/ultrafiltration (UF in the following) membrane operated as MBR. It was maintained at 20 ±1°C in a thermostatic room (Fig. 1). The anaerobic biofilm has a total volume of 2.7 L; it was packed up to a volume of 1.4 L with Biomaster BCN 012 KL (Amitec, Italy). The packing material has a cylindrical shape (12.5x12.5x12.5, LxWxH) with a cross separation inside and is made of polyethylene with density of 0.95g/cm^3, protected area of 500 m^2/m^3 and void degree of 86% (data from the supplier); a liquid volume of approximately 200 mL above the packing material maintained the biofilm always completely submerged. The void volume of the biofilm resulted in 1.2 L.

The working volumes of the anoxic and aerobic tanks were 1.0 and 1.1 L, respectively. Internal recycle was carried out using a peristaltic pump (Watson Marlow 403U/R1) at a constant flow rate of 5.0 mL/min for the entire duration of the study. A second peristaltic pump (Watson Marlow 401U/D1), controlled by a level sensor in the reactor, was used for feeding. A hollow fibre membrane module (ZW1, Zenon, Italy) with a membrane filtration area of 0.047 m^2 and nominal porosity of 0.04 μm (data from the supplier) was immersed into the aerobic tank. Membrane filtration was carried out using a programmable piston pump (Ismatec, Cole-Parmer, USA) whereas aeration was obtained using an aquarium blower (flow at approx 80 L h-1). To reduce fouling, the membrane

was operated alternating cycles of 3 and 2 min of filtration and relaxation, respectively; excluding the no-working days, back wash (15-20 min) was manually carried out daily.
The transmembrane pressure was monitored using a digital gauge (Cole-Parmer, USA). The membrane was removed and cleaned under tap water spray every approximately 50 days.
The hydraulic retention time and the solids retention time were maintained at about 1 and 80-100 days, respectively.

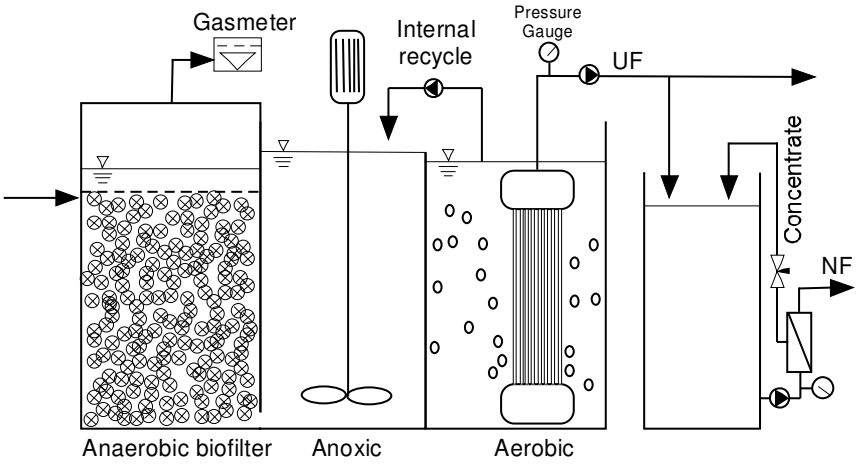

Figure 1. Schematic diagram of the bench-scale reactor.

Inoculum and Textile Wastewater

Before the beginning of this study, the reactor was operated with synthetic wastewater for approximately 3 months: more details of the start-up are reported elsewhere. [18] Then, the reactor was fed with real wastewater collected approximately every 20-30 days, in a textile factory located in western Slovenia. The wastewaters were sampled on the textile machineries outflows, and not on the main factory wastewater outflow, in order to avoid the most polluted streams (e.g. bleaching, dye batch). However, this caused even more variations of the wastewater characteristics. Therefore, the wastewater samples were mixed at different amounts every a few days before feeding the reactor in order to decrease the variability and achieve COD, nitrogen concentrations and colour in the

typical range of the textile wastewaters. When the resulted wastewater pH was above 10, it was corrected using diluted H_2SO_4.

Biomass acclimation to the real textile wastewater was achieved in approximately 15 days, mixing a synthetic wastewater [18] with the raw wastewater and gradually decreasing the amount of the synthetic one.

Nanofiltration

NF was performed by the bench equipment GE/Osmonics Sepa™ CF II Med/High Foulant System. Osmonics plane DL membranes with a filtration area of 140 cm^2, molecular weight cut-off of 150-300 Da, were used applying a pressure of 500 kPa. The tests were carried out in batch mode on samples of 2-3 L, filtering up to a concentrate volume equal of 20% of the initial volume (i.e. filtering 80% of the sample volume); permeates chemical characterisation was carried out on the entire filtrate volume (i.e. not at the different volumetric concentration factors). The samples treated by NF were constituted by portions of the effluent of the biological and MBR treatment (Fig. 1).

The fluxes were manually measured and were normalised to 20°C.

Analytical Methods

Chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl (TKN), ammonia, nitrite and nitrate nitrogen were measured according to Standard Methods. [27] Total organic carbon (TOC) was measured using a Shimadzu TOC-VCPH analyzer.

Dissolved oxygen (DO) and pH were measured using Crison probes and instruments.

Colour was estimated by colorimetric scan between 400-750 nm in 1 cm-length cells: the results are reported as mean values of the reference absorbance range. Colour was measured on filtrate samples. Filtration was performed using Whatman GF/C glass microfiber filters.

An estimation of the presence of aromatic amines was obtained according to the colorimetric method proposed by Norwitz and Keliher [28] using sulfanilic acid as standard. Due to the residual colour of the analysed samples and to the unknown formed aromatic amines, a quantitative estimation was not possible but the significant increase of

the absorbance after the addition of the colorimetric reagents was interpreted as the presence of aromatic amines.
Volatile fatty acids (VFAs) and biogas composition were measured by gas-chromatographic techniques. [18]
Biogas production was measured using an homemade wet-tip gasmeter.

4.3. Results and discussion

The biological reactor was operated for almost 4 months treating real textile wastewaters. Due to the moderate membrane fluxes applied, no severe fouling was observed. Moreover, due to the relatively (for the small aerobic tank) intense aeration applied in order to prevent membrane fouling, dissolved oxygen concentration in the aerobic tank was always above 5 mg/L.

Biological Treatment - MBR

The wastewater showed the typical high variability of the textile effluents. Moreover, high variation of the characteristics during wastewater storage was observed although it was maintained at 4°C; in particular, a significant colour decrease in relation to a slight coagulation was observed (probably due to microbial activity under anaerobic conditions).
The textile wastewater samples were usually highly coloured with maximum absorbance of 0.52 (mean value within the 400-750 nm wavelength range), with the highest pick of 1.801 at 494 nm. Although the wastewater variability, the biological system usually showed good colour removal (Fig. 2). In fact, the average absorbances were 0.206±0.13 (mean ± standard deviation) and 0.062±0.02 for the wastewater and the treated effluents, respectively. Moreover, it is noteworthy that, although the high influent wastewater colour variability, the treated wastewater presented a more stable colour with the average absorbance that was, with a few exceptions, below 0.1.

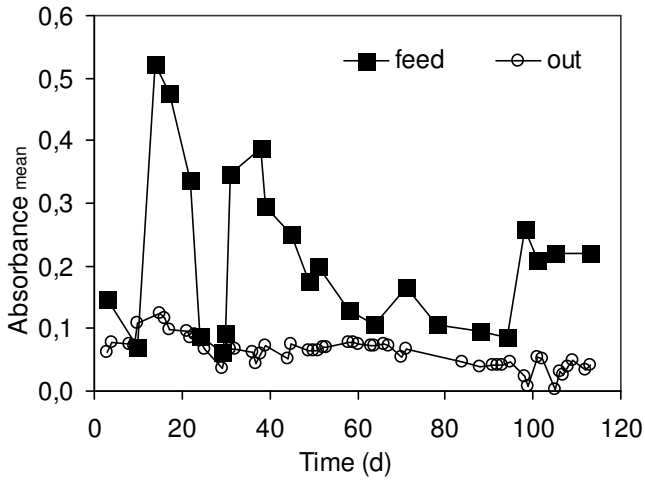

Figure 2. Mean absorbance of the textile wastewater and the ultrafiltrate.

Due to the different dyes used in the textile dyeing processes, the wastewater showed the maximum absorbance at different wavelengths. On the contrary, the permeate usually presented the maximum absorbance at wavelength comprised between 400 and 420 nm indicating a more refractory behaviour of the organics that absorb light at these wavelengths or to the release of organic matter by the biomass (e.g. due to biomass decay processes). As expected, [2] colour removal mainly took place under anaerobic conditions. The anaerobic treatment caused the formation of aromatic amines (qualitative determination), which were also refractory to the anoxic and aerobic conditions and therefore were also detected in the ultrafiltrate (data not shown). Note that, as described in the materials and methods section, quantitative determination of the formed aromatic amines was not possible due to the residual colour of the samples. The results, thus, confirm that the aromatic amines can be recalcitrant under aerobic conditions. [11,16-18]
The reactor was fed with raw textile wastewater with variable COD content. Although the variation of the COD load, the system showed a general good COD removal; in fact, the effluent COD concentrations were always below 96 mg/L with an average value of 54±13 (Fig. 3). Most of the textile organic matter was removed under anaerobic conditions. Figure 3 shows that with the increase of the organic loading rate, also the anaerobic COD values increased; on the contrary, the effluent COD values were much more stable. Therefore, the results confirm the effect of the membrane on the improvement of the biological wastewater treatment stability. [22, 23, 29]

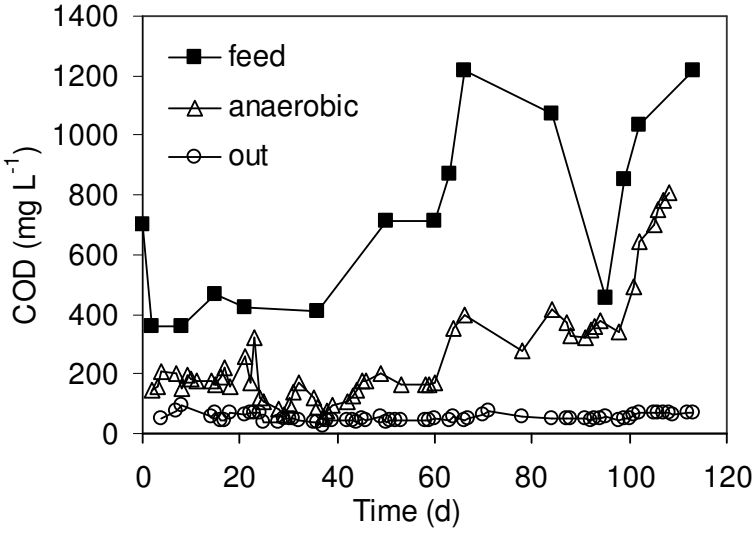

Figure 3. COD trends of the textile wastewater, the anaerobic biofilm effluent and the ultrafiltrate MBR.

The anaerobic COD removal was accompanied by a slight biogas production which was also rather variable (Fig. 4). The biogas variability was also previously observed in the same system treating synthetic wastewater [18] and was explained by the very low anaerobic vessel volume and therefore the possible presence of liquid short-circuiting. In addition, the high wastewater variability may also have increased the biogas production variability, likely having also some inhibitory effect on biomass activity; for example, the high biogas production rate measured on day 65 (Fig. 4) corresponds to a high influent COD concentration and low colour (dyes concentration). Since the biological dye removal depends on the availability of electron donors (at least for azo dyes) as the biogas production, the two biological processes are in competition for the biodegradable organic matter.

Due to the low organic loading applied to the anaerobic biofilm compared to the typical anaerobic digestion processes, the concentrations of the measured VFAs (data not shown) were always very low (below 20 mg/L), with only a few exceptions where acetic acid concentration approximated the value of 40 mg/L.

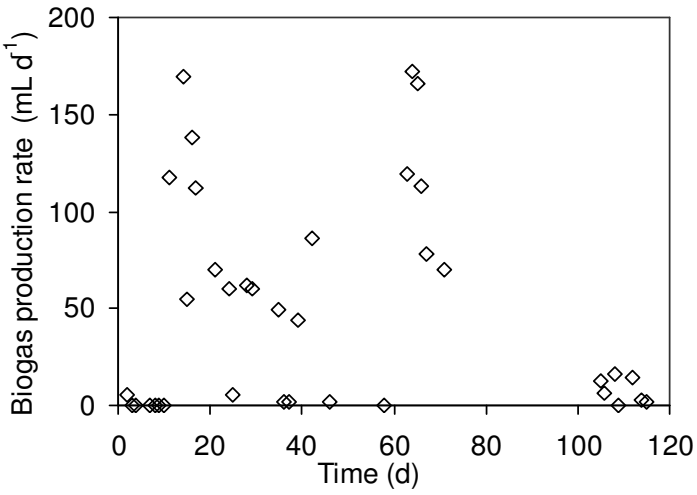

Figure 4. Biogas production rate in the anaerobic biofilm.

pH values also showed high variability (data not shown) that were related to the influent variability and to the biological processes in the system. Nevertheless, the pH values varied between 7.0 and 8.5 with an unique exception on experimental day 65 where the anaerobic pH decreased to 6.3 probably due to the increased loading rate; in fact, the same sample also presented the highest VFAs concentration.

The solids concentrations stabilised at the values of 7.7±2.8 and 5.0±2.0 g/L for TSS and VSS, respectively. Due to the presence of the ultrafiltration unit, solids in the effluent were absent. Nitrogen compounds are usually present at relatively low concentration in textile wastewater. However, urea is sometimes used as moisture-retaining auxiliary chemical in textile dyeing and printing processes. As the textile factory where the samples were collected uses urea in the printing processes and this process constituted a significant water flow in the factory (at least when these wastewaters were sampled), ammonia showed high concentration in some samples. Because the wastewater samples from different textile processes were mixed before being fed to the bench scale reactor, they presented a significant amount of nitrogen. Therefore, the processes were also tested in order to evaluate the nitrification and the nitrogen removal processes (denitrification).

TKN concentrations in the mixes of the wastewater samples results in an influent N concentration of 35±8 mgN/L (Fig. 5), with a significant variability (19-49 mgN/L). Although the biomass had been acclimated to synthetic textile wastewater containing azo

dyes [18] and to the real textile wastewater for 15 days before this study (see material and methods), a significant initial nitrification inhibition was observed. In fact, effluent ammonia concentration was always above 10 mgN/L during the first 20 days of the study, with a nitrification efficiency comprised between 17 and 63%. Thereafter, the nitrification efficiency increased significantly, achieving values higher than 95%, with the exception approximately after two months of operation when a new partial ammonia oxidation inhibition was observed. The results, thus, confirm that although a partial nitrification inhibition can be observed treating textile wastewater, significant nitrogen removal can be achieved after an adequate acclimation period.

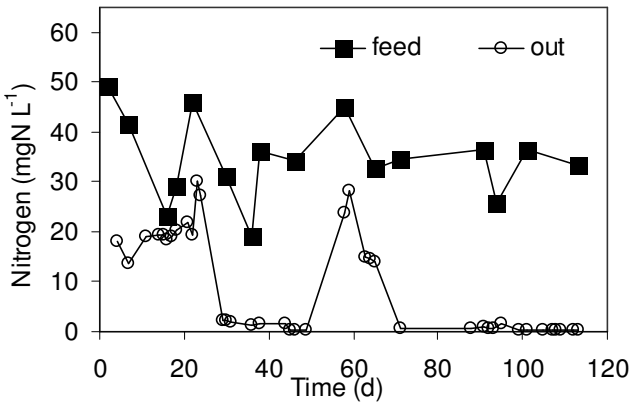

Figure 5. Influent TKN and effluent ammonia concentrations of the MBR.

The denitrification process suffered the variability of the ammonia and COD loading and of the nitrification efficiency resulting in very variable effluent nitrate concentrations (Fig. 6). In particular the system seems to be not very effective in removing nitrate when complete nitrification occurred and thus high effluent nitrate concentrations (even higher 20 mgN/L) were observed. Moreover, although the influent COD concentration seems to indicate an adequate COD/N ratio for complete nitrogen removal, the anaerobic biofilm significantly decreased the organic matter (Fig. 3) available for the denitrification process; as a result, high nitrate concentrations were also measured in the anoxic vessel (up to 18 mgN/L) and nitrogen removal sometimes presented values as low as 20-25%. On the contrary, nitrite concentrations were always below 0.1 mgN/L both in the anoxic and oxic vessels.

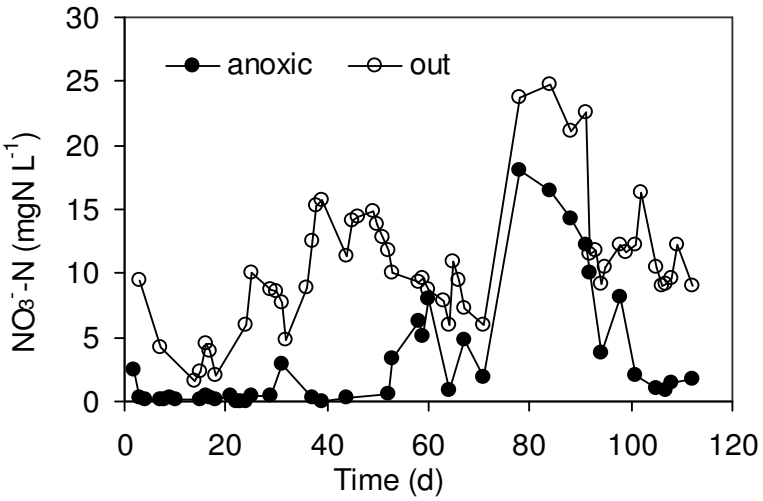

Figure 6. Anoxic and effluent nitrate concentrations of the MBR.

Nanofiltration

Textile industry requires high quality water for most of the fabric production processes. As a results, biological and UF wastewater treatments are not usually capable of producing the appropriate water for reuse. [30]

In this study, four samples were collected in four different experimental periods after MBR treatment.

Table 1 shows that the NF treatment further improves the water quality. The organic matter removal measured in this study ranged from 50 to 80% that is lower than results of some other studies where removals even higher than 90-95% have been reported (e.g. Alcaina-Miranda et al. [21]; however, it should be highlighted that the COD load applied to NF treatment was already relatively low due to the good performances of the MBR system.

Due to the high bivalent ions retention efficiency of the membrane used (96% MgSO4 rejection, data from the supplier), approximately 60-70% conductivity removal was obtained. The conductivity removal in NF treatment greatly depends on the salts used during the dyeing process: therefore, the relatively high conductivity removal measured in this study is related to the Na2SO4 presence in the wastewaters.

Although the MBR system was quite effective in colour removal (Fig. 2), the effluent was usually still yellowish. NF treatment proved to be also rather effective in removal of this biologically refractory colour (Tab. 2): in fact, a colour removal of 70-90% was achieved at the wavelength where the maximum absorbance was measured (comprised in the range of 400-410 nm), therefore almost completely removing the apparent colour (visual observation).

The results confirm the good efficiency that can be obtained using nanofiltration after MBR treatment. However, although the relatively stability of the NF treatment, the removal efficiencies were rather variable: De Florio et al. [25] previously demonstrated that not only the wastewater characteristics are highly variable but the pollutant removal efficiencies may also greatly depend on the type of the wastewater origin (i.e. the textile industry process, e.g. dyeing, scouring).

The NF treated water was tested in some typical textile processes, revealing very promising possible reuse in the factory; for example, no detectable differences in the dyeing process between the recycled and the softened freshwater used by the factory. [31]

Table 1. Main parameters measured in the MBR effluent (UF) and after NF treatment.

Parameter [Unit]	*Sample 1*		*Sample 2*		*Sample 3*		*Sample 4*	
	UF	NF	UF	NF	UF	NF	UF	NF
COD [mgO_2/L]	58	<15	75	22	56	39	68	35
TOC [mgC/L]	20	4	25	6	19	10	29	16
Conductivity [µS/cm]	1350	400	2220	810	1780	550	1830	610
Abs_{mean}* [-]	0.07	0.01	0.05	0.01	0.06	0.01	0.04	<0.01
Abs_{max}** [-]	0.29	0.02	0.18	0.05	0.18	0.02	0.10	0.02

*mean absorbance in the range 400-750 nm; **value measured at the wavelength where the absorbance were maximum (usually in the range 400-410 nm)

Although a significant flux decline was sometimes observed filtering (NF) the MBR effluents (occasionally even as low as 50% of the flux measured with tap water), due to the very small volumes of the samples and of the used filtration apparatus, filterability should be confirmed in more significant scale.

4.4. Conclusion

The study demonstrates that a system comprising an anaerobic biofilter and an anoxic-aerobic MBR is suitable for textile wastewater treatment. COD removal was usually higher than 95%. The use of an anerobic biofilter also allowed good colour removal which reached values usually higher than 70%. Nevertheless, because of the significant COD removal of the anaerobic biofilter (approx 60%), the available organic matter arriving into anoxic conditions was not always enough to assure complete nitrogen removal. Moreover, treating real wastewater sometimes a partial nitrification inhibition was observed.
The NF of the effluent of the biological and UF treatment allowed further COD, colour and salt removal allowing the production of water with characteristics suitable for reuse inside the textile factory.

Acknowledgments
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4.5. References

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Chapter 5

DECOLOURISATION OF TEXTILE WASTEWATER IN A SUBMERGED ANAEROBIC MEMBRANE BIOREACTOR*

Abstract

Azo dye decolourisation can be easily achieved by biological reduction under anaerobic conditions. The aim of this study was to evaluate the applicability of submerged anaerobic membrane bioreactors (SAMBRs) for the decolourisation of dyeing wastewater containing azo dyes. The reactive orange 16 was used as model of an azo dye. The results demonstrated that very high decolourisation (higher than 99%) can be achieved by SAMBRs. Although decolourisation was not significantly influenced by the azo dye concentrations up to 3.2 g L^{-1}, methane production was greatly inhibited (up to 80-85%). Since volatile fatty acids accumulated in the treatment system with the azo dye concentration increase, methanogens seem to be the most sensitive microbial populations of the anaerobic ecological community. The results demonstrated that anaerobic process combined with membrane filtration can deal with highly concentrated wastewaters that result from stream separation of industrial discharges.

Keywords*:* decolourisation, azo dye, anaerobic digestion, membrane bioreactor, high strength textile wastewater

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5.1. Introduction

Among the different classes of colorants, the azo dyes are the most abundant (O'Neill et al., 1999); they are characterised by one or more nitrogen-nitrogen double bond (-N=N-) called azo group. Dyes are used in different industrial sectors, among which the textile is one of the most important. The textile industry is also one of the most water-consuming sectors. Moreover, several auxiliary chemicals (e.g. salts) are usually added during the dyeing processes, resulting in wastewater with complex and variable characteristics (O'Neill et al., 1999).

The removal of azo dyes from wastewater can be accomplished with physical, chemical or biological processes, but the biological ones are usually preferred because they are cheaper and environmentally friendly. The azo bound is usually stable to aerobic bacterial biodegradation, whereas it is reduced under anaerobic biotreatment with the elimination of colour (Bonakdarpour et al., 2011; Carliell et al., 1995; Dos Santos et al., 2007; Pandey et al., 2007; Van der Zee and Villaverde, 2005). During anaerobic biological treatment, azo dye reduction results from the combination of biotic and abiotic reactions (van der Zee et al., 2003). Biotic processes involve the azo bond reduction via enzyme-mediated reactions, while abiotic azo dye decolourisation results from purely chemical reactions with reductants (e.g. sulphide) which are present in the anaerobic mixed liquor. Although both biotic and abiotic reactions might take place under anaerobic conditions, the biological ones seem to be more important for colour removal (van der Zee et al., 2003).

Azo dye decolourisation has been studied using both pure or mixed cultures; although promising results have been obtained using pure cultures (Ghodake et al., 2009; Parshetti et al., 2010), these seem to be not easily applicable at full scale for real wastewater treatment due to the presence of autochthonous microorganisms.

Over the last decades, several biological processes have been successfully applied to anaerobic azo dye decolourisation (Cervantes and Dos Santos, 2011). Most of the high rate anaerobic processes for wastewater treatment use biofilm or granular sludge to achieve a high biomass concentration. When biofilm or granulation cannot be easily achieved (e.g. high suspended solids concentration in the wastewater), membrane separation could represent an alternative to obtain high biomass concentration in the reactor and therefore maintain high treatment rate (Liao et al., 2006).

Membrane bioreactors (MBRs) have been successfully applied for municipal and industrial wastewater treatment. Two different configurations of MBRs have been applied: external, where the membrane modules are located outside the bioreactor, and submerged where the membranes are directly immersed into the reactor tank. Over the last two decades the submerged MBRs have accounted for almost the totality of the full- and pilot-scale applications. The majority of the applications of submerged MBRs in wastewater treatment are in aerobic processes where aeration is also used to create the cross-flow along the membrane to control the fouling processes (Meng et al., 2009). To reduce membrane fouling in submerged anaerobic MBRs (SAMBRs), the produced biogas can be recirculated and used instead of air bubbling of aerobic submerged MBRs (Liao et al., 2006).

Anaerobic MBRs (AMBRs) have recently received a great deal of attention from researchers (Liao et al., 2006). Most of the AMBRs in wastewater treatments have used the external configuration although over the last few years there has been increased research into SAMBRs (Gimenez et al., 2011; Hu and Stuckey, 2006; Jeison and Van Lier, 2008; Van Zyl et al., 2008). Since the energy requirement for submerged MBRs is usually much lower than for the side stream ones, at least for aerobic systems (Meng et al., 2009), application of SAMBRs could represent a further improvement in the energy balance of the wastewater treatment plant.

As stated above, the textile industrial sector is characterised by the generation of different wastewater streams with very high differences in pollutant concentrations; usually these streams are mixed together in order to obtain low to medium concentration wastewaters (O'Neill et al., 1999). On the contrary, in the context of water saving and reuse, it might be advantageous to treat separated high-strength wastewater streams instead of a mixture of several streams presenting low to medium strength. Therefore, anaerobic processes could be very effective for the treatment (or pre-treatment) of rather highly concentrated industrial wastewater resulting from streams separation in the textile factory.

The aim of this study was to evaluate the applicability of SAMBRs for the decolourisation of textile wastewater containing azo dyes. This work aimed to evaluate the anaerobic processes for the (pre)treatment of rather highly concentrated industrial wastewater generated by water management strategies that maintain separated streams in the textile factory. The reactive orange 16 (RO16) was used as model of azo dye.

5.2. Methods

Experimental set-up

The study was conducted using a laboratory-scale SAMBR (Fig. 1). The reactor had a total volume of 21.6 L (length x height x depth, LxHxD: 24x90x10 cm), a working volume of 11.4 L and was operated at 35±1°C in a thermostatic cabinet. The volume of the headspace was of approximately 10 L to prevent foaming problems that could be caused by biogas recirculation. A flat sheet ultrafiltration membrane module (Kubota®) of mm 225x315x6 (LxHxD), filtration area of 0.12 m^2 and nominal pore size of 0.4 µm was directly immersed in the sludge. Sludge mixing and membrane scouring to control cake formation were obtained by biogas recirculation through a coarse diffuser located just below the membrane module. The biogas flux was approximately 20 L min^{-1} (all volumes are expressed at standard conditions), which results in a superficial velocity of 53 m h^{-1} (calculated considering the transversal area of the reactor). Biogas was recirculated using a vacuum/compressor pump (KNF) and gas flow was regulated using a variable area flowmeter (Cole-Parmer International). The membrane was operated by alternating filtration and relaxation (i.e. no backwash) as indicated by the supplier: for the present study 4 min of filtration and 1 min of relaxation was applied. The treated water was extracted from the membrane applying a vacuum with a programmable piston pump (Cole-Parmer International).

Solid retention time (SRT) was maintained at approximately 150-200 d by manual extraction of a small amount of sludge once or twice per week, while hydraulic retention time (HRT) was maintained at 2.5 d.

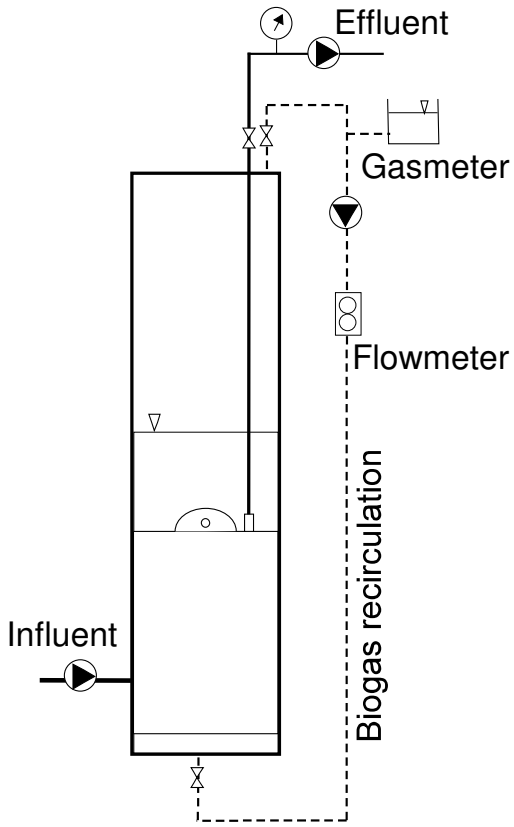

Figure 1. Schematic diagram of the laboratory-scale submerged anaerobic membrane bioreactor. Dashed and solid lines for gas and liquid pipes, respectively.

Feed and inoculum

The SAMBR was fed with synthetic wastewater containing sucrose (6.75 gCOD L^{-1}) as the only carbon source (except that of the azo dye) at an HRT of 2.5 d, resulting in a volumetric organic loading rate (OLR) of 2.7 gCOD L^{-1} d^{-1}. A salt solution composed of NH_4Cl (30 mgN L^{-1}), K_2HPO_4 (10 mgP L^{-1}), $NaHCO_3$ (3.5 g L^{-1}), $FeCl_2$ (0.35 mgFe L^{-1}) and Na_2MoO_4 (0.02 mgMo L^{-1}) was also fed to the reactor. The feed pH resulted at approximately 8.1. Since textile wastewaters usually have a significant conductivity (O'Neill et al., 1999), NaCl (400-500 mg L^{-1}) was included in the synthetic wastewater in order to increase the conductivity up to approximately 3 mS cm^{-1}. The reagents were dissolved in tap water. Filtration (and thus feeding) was completely switched off (maintaining biogas recirculation switched on) from d 73 and 88 because of the volatile fatty acids accumulation inside the reactor.

The reactive dye orange 16 (Sigma-Aldrich, 50 % dye content) was used as a model of the azo dyes typically used in textile factories. The dye concentration in the synthetic

wastewater was gradually increased (maintaining rather stable colour removal) from 0.06 g to 3.2 g L^{-1} (Fig. 2).

The lab-scale SAMBR was inoculated with granular sludge taken from a full-scale upflow anaerobic sludge blanket reactor treating effluents from a potato-processing factory situated in Bologna, Italy. Before this study, the bioreactor was operated for approximately eight months with synthetic wastewater with a composition similar to that used in this study but without dyes (Casu et al., 2012; Spagni et al., 2010a).

Analyses

Colour was measured by spectrophotometric absorbance at 494 nm where the dye presents its pick of absorbance. The dye concentration was estimated by a calibration curve.

Aromatic amines were qualitatively determined by spectrophotometric measurements after the diazotization-coupling reaction with N-(1-naphthyl)ethylenediamine according to Norwitz and Keliher (1982); sulfanilic acid, which has a chemical structure similar to one of the two amines expected from the azo dye degradation, was used as standard for the calibration curve. Since different aromatic amines react differently to the colorimetric reaction, the significant increase of the absorbance after the addition of the colorimetric reagents was interpreted as the presence of aromatic amines. The limited interference of the colour of the dye on that of the colorimetric complex was verified analysing their spectra.

Chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to the Standard Methods (APHA, 2005).

Sugars were measured using the phenol-sulphuric acid reaction using sucrose as standard (Dubois et al., 1956). pH was measured using Crison probe (pH 5202) and instrument (pH-meter Basic 20).

Volatile fatty acids (VFA) were determined by using a gas chromatograph (Dani 8510) equipped with a capillary column (DB-FFAP, 30 m x 0.53 mm x 1.5 µm), a flame ionisation detector and using hydrogen as carrier gas. Biogas composition was measured by a second gas chromatograph (Dani 3865) equipped with a stainless steel column (Haye-sep"Q" 80/100, 2 m x 1 mm), a thermal conductivity detector and using nitrogen as carrier gas.

Biogas production was measured using a homemade wet-tip gas-meter.

Transmembrane pressure (TMP) was measured using a digital gauge (Cole-Parmer International).

5.3. Results and discussion

The laboratory-scale SAMBR was operated for almost four months with increasing azo dye concentration from 0.060 to 3.2 g L^{-1} (Fig. 2).

During the experimental time, reactor pH was almost at neutral value (7.1±0.3; mean ± standard deviation), with slight decreases only when VFAs accumulated; although the significant VFA accumulation that was sometimes observed, the pH never decreased below 6.6. Biomass concentration (estimated as sludge TSS and VSS) also remained rather stable (20.3±1.9 and 14.5±1.8 g L^{-1} for TSS and VSS respectively) with a downward trend during the experimental campaign (data not shown). Due to the presence of the ultrafiltration membrane, suspended solids in the effluent were completely absent.

Due to the relatively low permeability of the membrane under anaerobic conditions (Spagni et al., 2010a), a very low membrane flux was applied, resulting in a HRT of 2.5 d. As a result of the very low membrane fluxes applied (approximately 2 L m^{-2} h^{-1}) and the relatively low experimental duration (for evaluating membrane fouling processes) no significant membrane fouling was observed and, thus, TMP values were always below 40 kPa (TMP limit value from the membrane supplier).

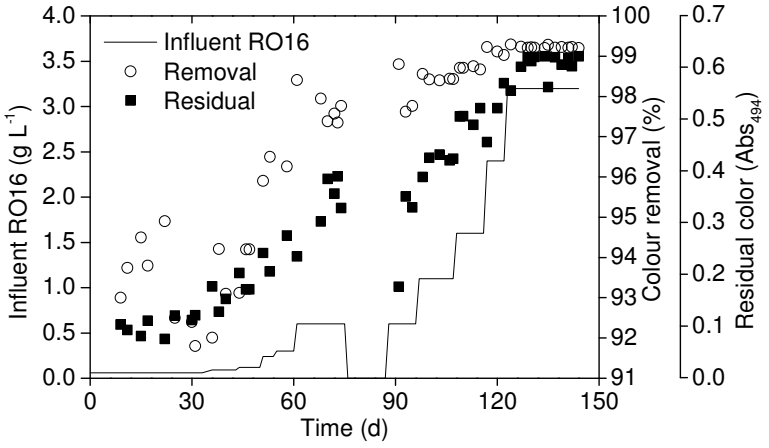

Figure 2. Influent azo dye concentration, colour removal and residual colour in the effluent.

Azo dye removal

Contrary to other studies where the treatment of textile wastewater containing relatively low amount of colourants (e.g. Carvalho et al., 2008, Grilli et al., 2011; Spagni et al., 2010b; Wijetunga et al., 2010), this work aimed to evaluate the anaerobic processes for the treatment of rather concentrated industrial wastewater.

The reactor was fed with a constant (60 mg L^{-1}) and rather low RO16 concentration for approximately one month in order to encourage biomass acclimation to the azo dye (Fig. 2).

Azo dye removal (measured as colour removal) was immediately very high with values higher than 90% and gradually increased with biomass acclimation and with azo dye concentration increase. During the first two months, applying a low azo dye load, the colour removal was comprised between 91 and 95%.

Although colour removal was very high, with the slight increase of the influent azo dye concentration operated during the first two months, VFAs accumulated in the reactor (see section 3.3), causing a partial inhibition of the anaerobic processes. Therefore, after 73 d of experimentation, the filtration was switched off (causing the feeding stop) in order to allow the anaerobic process to stabilize. Since (on experimental day 88) a low VFAs concentration was measured after the reactor was switched off, filtration was restarted. Because the process stabilised very rapidly, the azo dye influent concentration was then continuously increased up to the maximum value of 3.2 g L^{-1}. Although the VFAs showed some increases even after the first two months of operation, their concentrations were always much lower than during the acclimation period, demonstrating that anaerobic biomass can deal with rather high azo dye concentration after a sufficient period of acclimation. The biomass acclimation was also confirmed by changes in the microbial community that were qualitatively observed by microscopic examination as reported elsewhere (Cellamare et al., 2009).

The colour removal increased (up to the maximum values of 99.2%) with the increased influent azo dye concentration demonstrating the effectiveness of the proposed anaerobic treatment system (Fig. 2).

Abiotic colour removal was assessed filtering the synthetic wastewater using a new membrane identical to that used in the reactor. The filtration of the synthetic wastewater without the presence of biomass accounted for a colour removal lower than 10 %

confirming that the azo dye removal was mainly due to microbiological azo dye reduction. Although azo dye absorption on biomass cannot be excluded, its effect should be negligible on the total removal because of the high azo dye load and the long SRT (150-200 d).

Although the effectiveness in colour removal and the increased colour removal with the increased azo dye influent concentration, the residual colour of the permeate also increased producing a water that was still rather coloured (Fig. 2). Due to the high azo dye load, the colour removal was still higher than 99 %; in fact, the highest colour measured in the permeates of the SAMBR corresponded to RO16 concentration of 30-40 mg L^{-1}.

Therefore, the results confirmed the decolourisation of the RO16 under anaerobic conditions, as previously reported (Jadhav et al., 2010; Kapdan and Oztekin, 2003). However, this study also demonstrated that high anaerobic biomass concentrations, as obtained in the studied SAMBR, can deal with higher azo dye loads than those usually applied before and therefore the proposed technology can be applied for the treatment (or pre-treatment) of wastewater produced by the separation of highly concentrated streams in the textile factories.

Although other authors also reported very high colour removal using other high-rate anaerobic reactors, the applied azo dye concentrations were usually much lower than those used in this study (Carvalho et al., 2008; Cruz and Buitron, 2000). For instance, Cruz and Buitron (2000) treated the dispersed blue 79 by an anaerobic biofilter up to the concentration of 114 mg L^{-1} whereas Carvalho et al. (2008) used an upflow anaerobic sludge blanket for the treatment of the acid orange 7 up to 300 mg L^{-1}. Much lower concentrations (100-300 mg L^{-1}) were also tested in a very extensive study using granular sludge on 20 different azo dyes (Van der Zee et al., 2001).

Carbon metabolism

The COD removal had an opposite trend with respect to colour removal (Fig. 3). In fact, COD removal reached the highest values during the acclimation period. The high COD removal during the acclimation period could be due to the operating conditions applied before the addition of the azo dye; the reactor was indeed operated for almost eight months treating synthetic wastewater with even higher OLR than that applied in this study without high VFAs accumulation (Casu et al., 2012). The increase of the azo dye load

caused a serious deterioration of the COD removal after approximately two months of operation (Fig. 3). COD removal decreased from 94-95 % of the first week, to approximately 10 % with the first increase of the azo dye concentration to 600 mg L^{-1}. After the filtration was switched off, COD removal recovered (Fig. 3). Thereafter, although the azo dye concentration was significantly increased (when the feeding was switched on again), the COD removal was never so severely inhibited as during the first 73 d of the experiment. Moreover, even if other cases of VFAs accumulation were still observed with the increased RO16 load, their concentrations were always below 1.2 g L^{-1} (Fig. 4). Furthermore, the increase of the azo dye influent concentration affected the effluent COD concentration (and thus the COD removal) likely due to the presence of the formed aromatic amines. Consequently, over the last two experimental months the effluent COD concentration increased from approximately 0.8-1.0 to 4.0-4.5 gCOD L^{-1} and, as a result, the COD removal decreased from approx. 90-95% to 55-60 % at the beginning and the end of the study, respectively (Fig. 3). Due to the presence of the membrane that completely retained the suspended solids inside the reactor, the effluent COD was always mainly composed of the metabolites of the fermentative processes (i.e. VFAs and aromatic amines).

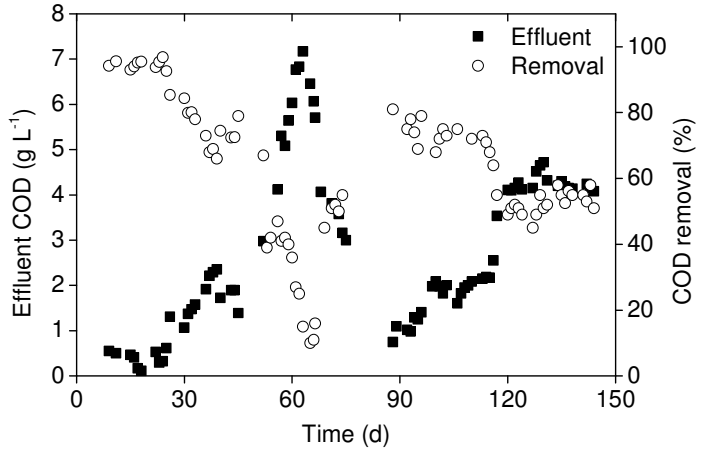

Figure 3. Effluent COD and COD removal

The effluent COD increase was mainly due to VFAs concentration build-up, whereas the sugars concentrations were always below 10 mg L^{-1}. Therefore, the VFAs accumulation

seemed to be mainly related to the presence of the azo dye (or the formed aromatic amines) that, thus, appeared to inhibit the methanogenic biomass (at least if not acclimated). The complete sugars consumption with the accumulation of VFAs confirms the methanogenic bacteria as the most sensitive microorganisms in the anaerobic digestion process dealing with the presence of azo dyes.

It is of note that, together with acetic acid, propionic acid also accumulated in the reactor, and, on experimental d 60 the concentration of the latter was even higher than that of the former (Fig. 4). The propionate accumulation in anaerobic reactors has been identified as an indication of possible overload or inhibition of anaerobic fermentation. In fact, the propionate metabolism has been correlated with low methane production rate and, hence, its accumulation has been proposed as a monitoring and control parameter in anaerobic digestion (Boe et al., 2008; Pind et al., 2003). Other VFAs (butyric and valeric) were also detected during the study, but their concentration were always below 500 mg L^{-1}.

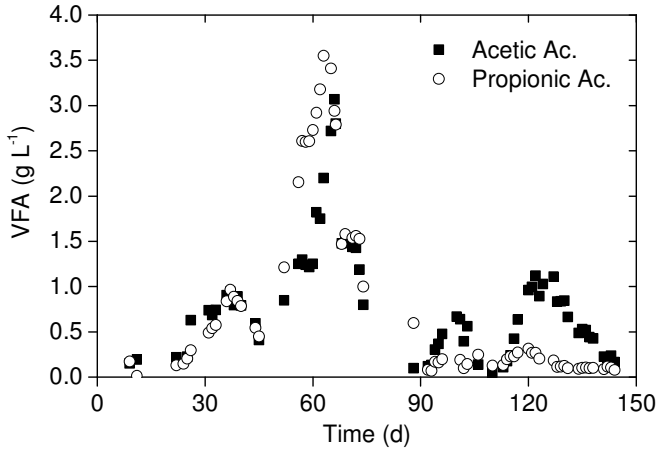

Figure 4. Concentration of the two main volatile fatty acids measured in the effluent.

It has to be highlighted that a significant fraction of the effluent COD was likely composed by the aromatic amines that were produced by the azo dye cleavage and that their concentration increased with the increased azo dye concentration load (data not shown). Therefore, the formed aromatic amines resulted refractory to anaerobic digestion

as already reported in the literature (Cervantes and Dos Santos, 2011; Dos Santos et al., 2007; Pandey et al., 2007).

The azo dye also severely affected the biogas production. Even though the SAMBR showed good biogas production during the acclimation period, methane production rate significantly decreased with the increase of the azo dye load (Fig. 5).

Methane production, although rather variable, presented rates up to 10 L d^{-1} during the first 30 d of experimentation, when the azo dye concentration remained at 0.06 g L^{-1}. The estimated gas yield was of 320-340 mL CH_4/g COD removed, that was in agreement with anaerobic reactors operating under non-inhibitory conditions.

Thereafter, CH_4 production rate remained at 8.0-8.5 L d^{-1} when the azo dye concentration was gradually increased ten-fold to 0.6 g L^{-1}, therefore showing a slight inhibition of 15-20 %. With VFAs accumulation after two months of experimentation, negligible biogas production rates were measured. Over the last two months of the study, methanogenic activity slightly recovered although the azo dye concentration was increased to the maximum value of 3.2 g L^{-1}. However, it never recovered to the methane production rates measured at the beginning of the study: therefore, approximately 80% methane production inhibition was observed with the highest azo dye concentrations applied.

On the contrary, biogas composition was not significantly affected: methane accounted for 46-53 % of the biogas and CO_2 for the remaining amount. As expected, the highest biogas production variations were related to the variability of the COD removal (Van Lier et al., 2008).

Other researchers reported different effects of azo dyes on methane production and yield. For example, in the study of Carvalho et al. (2008) using a bench-scale upflow anaerobic sludge blanket (UASB) treating the azo dye acid orange 7, the biogas yields were not significantly influenced by the azo dye presence. In a very recent study again using a UASB reactor, Wijetunga et al. (2010) also did not find a significant effect of the azo dyes on biogas production. In addition, another recent study using an anaerobic biofilm reactor treating the azo dye RO16 also did not reveal a great effect on biogas production rate and yield (Spagni et al., 2010b).

On the contrary, severe inhibition of the methanogenic activity by azo dyes has sometimes been reported. Tan et al. (1999), for instance, found the azo dye mordant orange 1 very inhibitory to the methanogenesis. Similarly, a very inhibitory effect of the azo dye methyl orange in an anaerobic sequencing batch reactor was evidenced by Yu et al. (2011). It is of

note that this latter study (Yu et al., 2011) also showed the accumulation of VFAs in the reactor with the increase of the azo dye concentration.

The varying effect of the azo dyes on anaerobic methane production may be due to the different concentrations applied. In fact, low or no methanogenic inhibition was usually observed when low azo dye concentrations (usually lower than 200-300 mg L^{-1}) were applied (e.g. Carvalho et al., 2008; Spagni et al., 2010b; Wijetunga et al., 2010). On the contrary, significant methanogenic inhibition was observed when higher azo dye concentrations were treated as in this study. Moreover, the acclimation of the biomass seems to play in important role in the anaerobic processes in the presence of azo dyes. In fact, this study highlights that the anaerobic biomass can deal with increasing azo dye concentration when acclimation takes place. Therefore, the high inhibitory effect that was observed by other authors (e.g. Tan et al., 1999) treating low azo dye concentration (50 mg L^{-1}) could be related to an insufficient (or a complete lack) of acclimation.

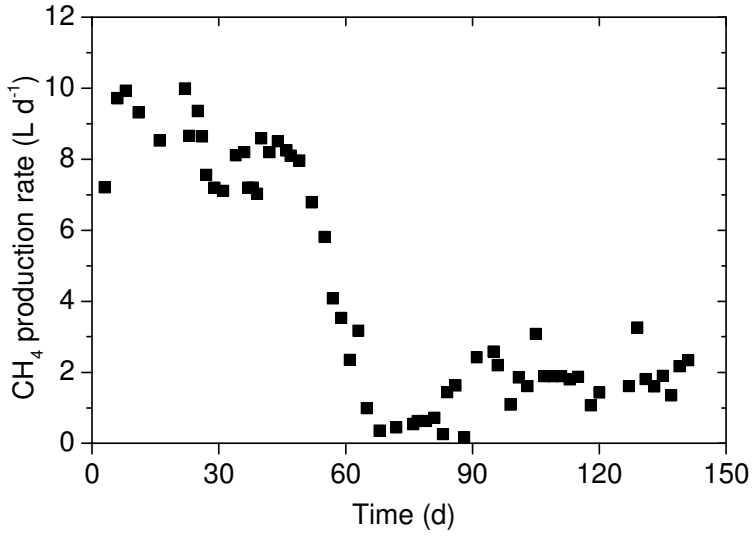

Figure 5. Methane production rate.

This study confirms the inhibitory effect of azo dyes on fermentative processes. Since literature data (as recently reviewed by Cervantes and Dos Santos, 2011) demonstrate that the increase of the azo dye concentration increases the inhibition of the methane production, and the inhibition ceases with the complete azo dye reduction, the effect of the azo dye on the methane fermentative processes seems mainly related to the competition between methanogenesis and azo dye reduction for the reducing equivalents (Cervantes

and Dos Santos, 2011). However, as already stated by other authors (Tan et al., 1999), the results of this study do not fully support this explanation. In fact, the azo-bound cleavage requires the transfer of four electrons (Dos Santos et al., 2007), resulting (for RO16 disodium salt, molecular weight of 617.54 g mol^{-1}) in the requirements of 0.052 g O_2 (as COD) per every g of RO16. Therefore, although a very high RO16 concentration was used in this study, the reduction of the added azo dye could maximally consume 2-3 % of the electron-equivalents available from the influent sucrose. Thus, the azo dye cleavage under anaerobic conditions seems to be also related to an easily reversible metabolic inhibition instead of the competition for the electron equivalents alone.

However, the molecular structure of the azo dyes and of the resulted aromatic amines seems to play a significant role on biological inhibition. In fact, the toxicity potential of the aromatic amines resulting from azo dye reduction is highly dependent on the position, type and number of substituents in the aromatic rings (Brown and De Vito, 1993). Therefore, the varying inhibitory effect observed in the literature could also be due to the different composition (i.e. different azo dyes and resulting aromatic amines) of the treated wastewater.

5.4. Conclusions

The results demonstrated that membrane bioreactors under anaerobic conditions can achieve a very high decolourisation of wastewater containing azo dyes. Moreover, the proposed technology can be applied to treat wastewaters containing high azo dye concentrations and, thus, can deal with wastewaters that are generated by stream separation in industrial factories (e.g. textile).

Although decolourization was not significantly influenced by the azo dye concentration increase (up to 3.2 g L^{-1}), methane production was greatly inhibited. Methanogens seem to be the most sensitive microbial populations and therefore accumulation of VFAs is expected when treating wastewater characterised by high azo dye concentrations.

Acknowledgements

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Chapter 6

STRATEGIES FOR WATER RECYCLING IMPLEMENTATION IN SLOVENE TEXTILE COMPANIES*

Abstract

This paper presents proposed solutions for water reuse implementation in two Slovene textile finishing companies.

As a first step a very complete database was obtained with the collection and characterization of all relevant water related textile production processes. On the one hand, data concerning water use, chemicals and energy were collected for all relevant production processes. On the other hand, all relevant batch discharges from each process were analyzed by measuring relevant ecological parameters. Afterwards textile effluents were classified in high and low concentrated, in view of their separate treatments and further reuse possibilities. The distinction between low and high concentrated effluents was based on effluents potential treatability by membrane and AOP technologies and their reusability in textile processes. As final step laboratory scale dyeing with recycled water and process water was realized with the aim of colour difference determination between normal process water and recycled water.

Keywords: textile finishing industry, water reuse, water scarcity

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6.1. Introduction

Sustainable water use becomes one of the most important issue in European water policy. Textile finishing industry is very water intensive. Wastewater is by far the largest waste stream in the textile industry. Scouring, dyeing, printing and finishing processes generate the majority of wastewater, as they require many rinsing sequences after each step.

For development of strategies for water treatment and reuse, complete characterization of water related textile process effluent streams is needed. When the characteristics of the separate streams/discharges are known, it can be decided which streams/discharges can be combined, to optimize treatability and suggest reuse options. Normally in textile finishing companies do not use any water reuse, while fresh high quality water is needed in all the production processes. The stringent environmental regulations and water scarcity in some European regions, forcing the textile industry to examine the potential for reusing the water from the textile wastewater streams. The choice of the treatment technology depends on the nature of the wastewater and also on the amount of annually used fresh water [1, 2].

A general quality standard for textile water to be reused it is quite difficult to define, because of the different requirements of each textile material and of the textile process applied and because of the different quality required for the final fabric. Parameters need to be considered for reuse was used as initial guidelines as suggested by different authors [3, 4].

In the present study after the complete chemical characterization, criteria for the effluent separation in so called low concentrated and high concentrated effluents, based on their treatability and further reusability were defined. Afterwards, simple reuse scenarios were proposed.

In these scenarios wastewater treatment technologies proposed are different combinations of membrane treatment by ultrafiltration/nanofiltration and advanced oxidation by UV/H2O2 for low concentrated waste streams. Treated samples were further used in so called ˝reusability˝ experiments where laboratory dyeing of cotton according to company dyeing procedures were realized. Characteristics of dyed cotton material were compared to the cotton material dyed with normal process water.

6.2. Results and discussion

All individual discharges connected to textile processes that are relevant on yearly basis were collected and characterized by physical-chemical parameters. Normally in textile SMEs machinery are used to carry out different production processes. For this reason the diversity of waste discharges characteristics could vary significantly.

The significant fluctuation of pollution level of waste discharges from different textile production processes is presented in Table 1 and Table 2.

Table 1. Pollution level of different textile discharges from individual processes

Sample	pH	Conductivity ($\mu S\ cm^{-1}$)	Turbidity (NTU)	COD ($g\ L^{-1}$)	TSS ($g\ L^{-1}$)	Absorbance		
						436	**525**	**620**
Dyeing yarn, reactive dyes, light	10.8	46900	9	792	94	0.49	1.91	0.32
Dyeing yarn, reactive dyes, dark	10.2	71900	0	3850	154	15.66	18.75	2.51
Dyeing fabric, foulard, reactive dyes, dark	13	69400	3	31960	1852	124.5	272.4	450.2
Fabric washing and bleaching	12.7	24100	200	32409	2850	0.89	0.56	0.39
Fabric bleaching, foulard	12.9	115200	200	61900	7629	2.85	2.54	2.37

Table 2. Ion content of different textile discharges from individual processes ($mg\ L^{-1}$)

Sample	Cu^{2+}	Mn^{2+}	Fe	Na^{+}	Ca^{2+}	Cl^{-}	SO_4^{2-}
Dyeing yarn, reactive dyes, light	0.602	0	0.026	12400	9.10	>3000	400-800
Dyeing yarn, reactive dyes, dark	5.75	0.0001	0.053	15000	7.25	-	400-800
Dyeing fabric, foulard, reactive dyes, dark	930.9	0	0.823	12000	0	-	-
Fabric washing and bleaching	0.155	0.067	0.639	3700	0	500	>1600
Fabric bleaching, foulard	0.277	0.357	0.303	19400	7.94	-	>1600

*measurement unit: ($mg\ L^{-1}$)

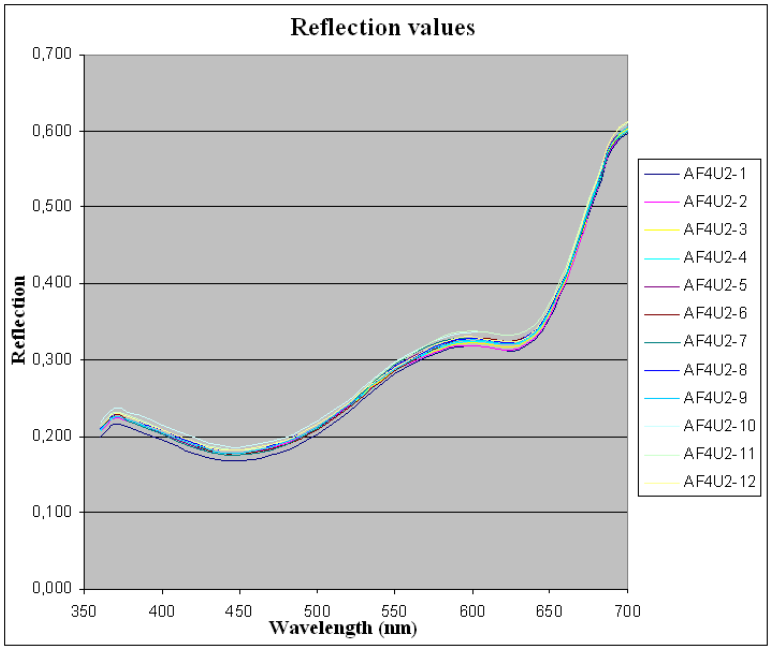
Particularly streams were very polluted expressing in high conductivity, absorbance, COD and total suspended solids. Other streams are not so concentrated and after proper treatment the quality of recycled water could be good enough to be reused. So called ˝low polluted streams˝ from different textile processes (i.e. dyeing, washing, rinsing) were

collected separately and treated by membrane techniques and AOP separately or with different combinations of both techniques. The more concentrated effluents and the concentrates produced by the membrane technologies should be treated with different technologies appropriate for so called ˝high concentrated˝ streams.
By proposed treatment procedures for low concentrated streams we have tried to achieve the following values of parameters need to be considered for water to be reused according to literature data:

pH: Almost all the authors agree on a pH required in the range of 6,5-8,0.
Conductivity: Most of the authors suggest maximum values in the range 1-2 mS/cm.
Suspended Solids: The authors suggest maximum values in the range 5-50 mg/L.
Turbidity: Only one author proposes a maximum value of 1.0 NTU.
Total COD: Maximum values for reuse range between 10 and 160 mg/L.
Colour: Most authors recommend the water should be colorless (roughly corresponding to an absorbance at each wavelength <0.01 cm-1).
Metals: According to what is suggested by most authors, to dyestuff producers and textile finishing company recommendations the following values are proposed: Iron 0,1 mg/L, Manganese 0,05 mg/L, Copper 0,05 mg/L.

According to the conclusions drawn from the analysis of the existing water and wastewater network and from the effluents characterization, simplified reuse network scenarios were designed. Scenarios are based on machinery separation and on effluents separation based on continuous monitoring of the effluents characteristics. In these scenarios wastewater treatment technologies evaluated are different combination of coagulation, UF, NF, AOP (UV/H2O2), MBR and evapoconcentration. To evaluate the effectiveness of proposed reuse scenarios simple laboratory scale dyeing experiments were performed using treated water with proposed treatment technologies.
Identical pieces of cotton fabric were put in dyeing vessels together with the samples of dye bath. Vessels containing fabric and dye bath were installed in the laboratory dyeing machinery that simulates the real condition of full scale dyeing. Dyed fabrics were washed and dried and in the end dyed material was evaluated by colour matching.
In general laboratory dyeing experiments using recycle water gave promising results in comparison to dyeing with normal process water.

As an example reflection curves of dyed material with recycled water obtained after different combinations of proposed treatment technologies are presented in Figure 1.



LEGEND:
1. discharge after washing treated with COAG. + UF (hollow fiber);
2. discharge after washing treated with UF (hollow fiber) + NF
3. discharge after dyeing treated with UF (hollow fiber) + NF
4. discharge after dyeing treated with COAG. + UF (hollow fiber);
5. discharge after dyeing treated with UF (spiral wonded) + NF
6. discharge after dyeing treated with UF (spiral wonded) + NF
7. discharge after printing mis treated with COAG. + UF (hollow fiber);
8. discharge after washing treated with UF (hollow fiber) + AOP
9. discharge after washing treated with AOP (1600 W, 8.3 mL $L^{-1}H_2O_2$; 30 min)
10. discharge after washing treated with AOP (1600 W, 4.5 mL $L^{-1}H_2O_2$; 30 min)
11. discharge after washing treated with AOP (1600 W, 4.5 mL $L^{-1}H_2O_2$; 30 min)
12. dyeing with process water.

Figure 1 Spectrophotometric verification of dyed cotton fabrics with recycled (samples from 1-11) and process water (sample 12)

6.3. Conclusions

Above described methodology could be carried out in all SMEs interested in wastewater reuse in the textile sector and in other industrial sectors characterized by a similar (weekly, monthly and yearly) variation of effluents discharges by production machinery. Separation

and segregation of waste water for treatment and re-use differs company by company and depends on different factors. Sometimes separation on machinery level is possible, in other companies it will only be based on the concentration of certain parameters. Criteria have to be developed for each case separately to decide on the best way of separation and segregation.

Recycling experiments in textile finishing companies, using treated water for the production purposes should be obligatory part of proposed treatment technologies testings to assure practical applicability of reuse treatment concepts.

6.4. References

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Chapter 7

STABILISATION OF BIODRIED MUNICIPAL SOLID WASTE FINE FRACTION IN LANDFILL BIOREACTOR*

Abstract

The biodrying process of solid waste is a pre-treatment for the bio-stabilization of the municipal solid waste. This study aims to investigate the fate of the municipal solid waste fine fraction (MSWFF) resulting from a biodrying treatment when disposed in landfills that are operated as bioreactors. Biodried MSWFF was apparently stable due to its low moisture content that slows down the microbial activity. The lab-scale anaerobic bioreactors demonstrated that a proper moisture content leads to a complete biodegradation of the organic matter contained in the biodried MSWFF. Using a pilot scale landfill bioreactor (LBR), MSWFF stabilisation was achieved, suggesting that the leachate recirculation could be an effective approach to accomplish the anaerobic biodegradation and biostabilisation of biodried MSWFF after landfilling. The biostabilisation of the material resulting from the LBR treatment was confirmed using anaerobic and aerobic stability indices. All anaerobic and aerobic indices showed a stability increase of approximately 80% of the MSWFF after treatment in the LBR. The similar values of OD7 and BMP stability indices well agree with the relationship between the aerobic and anaerobic indices reported in literature.

Keywords: Biodrying, municipal solid waste fine fraction, moisture content, biogas, mechanical-biological treatment.

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7.1. Introduction

Over the past decade there has been a growing interest worldwide to improve waste management. In Europe, for instance, this interest has resulted in efforts for identifying and developing new strategies to meet the "waste hierarchy" (prevention, reuse, recycling, recovery and disposal) for waste prevention and management (EC Directive 98, 2008).

Although waste management practices should primarily consider the reduction of waste production, a large fraction of the produced municipal solid waste (MSW) at least in Italy is still disposed of in landfills (ISPRA, 2011).

Mechanical-biological treatment (MBT) technologies combine mechanical processing such as size reduction with biostabilization treatment such as composting, anaerobic digestion or biodrying (Juniper, 2005). MBT plants are being used for treating waste with the aim of improving waste management through the production of stabilised material for landfilling or, preferably, of added-value products such as solid recovered fuel (SRF) or compost (Juniper, 2005).

A desirable option for waste management is the energy recovery as SRF that can meet the dual goal of reducing the disposal and producing an alternative energy source (Tunesi, 2011). SRF represents a renewable solid fuel that is prepared from non-hazardous waste in order to meet quality specification (Velis et al., 2010).

Biodrying is a treatment included in MBT technologies that is optimised for SRF production, opposed to other MBTs (i.e. those that include composting treatment) where the main objective is waste stabilisation (Velis et al., 2009). Biodrying is a relatively new technology for waste treatment (Velis et al., 2009) and a few studies have been only very recently published (e.g. Ragazzi et al., 2011; Tambone et al., 2011; Velis et al., 2012; Wagland et al., 2011; Zawadzka et al., 2010; Zhang et al., 2008; 2011). Biodrying reactors usually receive shredded unsorted MSW, where the treatment consists of the combination of thermal energy released during aerobic biological reactions with excess aeration, resulting in the drying of the waste (Velis et al., 2009; 2010). Additionally, the resulting dry material is afterwards screened in order to separate the oversize fraction characterised by high net heating value from the smaller fraction ("biodried MSW fine fraction", FF, MSWFF hereafter) that is characterised by low heating value (Tambone et al., 2011; Velis et al., 2009; 2010). Therefore, while the oversize fraction can be effectively used as SRF, MSWFF is usually disposed of in landfills. Since the biodrying treatment mainly reduces (or removes) the

moisture content of the waste, instead of being a real biostabilisation process through organic matter biodegradation, MSWFF disposal in landfills may cause "waste reactivation" and thus biogas emissions when the refuse moisture content is recovered by leachate production.

The management of a landfill as an anaerobic bioreactor is an approach to increase waste degradation and stabilization, methane production and management, and to reduce the life time of the landfill bioactivity. In the landfill bioreactor the refuse stabilisation is mainly optimised by moisture control through leachate recirculation. Increasing the moisture content and water flux through the refuse creates a favourable environment for organic matter biodegradation (Kumar et al., 2011; Reinhart et al., 2002). Therefore, pretreating the waste before landfilling and monitoring and controlling the waste moisture content are the two major options to optimize anaerobic MSW biodegradation and biogas production (Bayard et al., 2011; Benbelkacem et al., 2010).

This study aimed to investigate the fate of MSWFF derived from biodrying treatment when disposed in landfills that are operated as bioreactors. MSWFF stability was evaluated after moisture recovery by leachate addition. Thereafter, a pilot-scale reactor (of 1 m^3) was built to simulate the landfill bioreactors processes for the MSWFF degradation/stabilisation. The study allowed us to evaluate the biostabilisation that takes place in the landfill bioreactor due to a complete methanisation of the organic matter contained in MSWFF.

7.2. Materials and methods

7.2.1. MSWFF and landfill leachate

The experiment was performed on waste samples collected at a full-scale MBT plant located in Northern Italy that conducts bio-drying and refining for energy recovery and landfilling minimisation of MSWFF. The biodrying full-scale plant treats approximately 160.000 Mg y^{-1} of unsorted and shredded MSW. The resulting bio-dried material is thereafter mechanically treated by coarse grinding, size separation and deferrization. The sieved fraction (60-mm opening), that is disposed of in a landfill bioreactor, forms the MSWFF material used in this study, whereas the produced SRF is used in a cement kiln.

Approximately 400 kg of MSWFF were sampled and used for characterisation and for carrying out the anaerobic biotreatment experiments in laboratory- and pilot-scale reactors.

Moreover, leachate from the full-scale landfill bioreactor receiving the MSWFF was also collected for use in the lab- and pilot-scale reactors (see sections 2.2 and 2.3).
Sampling was performed according to the Italian rule UNI/TS (2004) and the samples were stored at 4°C before analysis.

7.2.2. Laboratory scale anaerobic bioreactors

Lab-scale reactors were used to evaluate the anaerobic stability index of MSWFF; moreover, the stability index was also evaluated after moisture increase by leachate addition.
The anaerobic stability index was measured by the biochemical methane potential (BMP) test up to 100 days, according to Owens and Chynoweth (1993) with minor modifications. The tests were carried out in two-litre pyrex-glass bottles, filled with approximately 1000 g of MSWFF, and rehydrated with leachate addition in order to obtain different moisture levels of approximately 200%, 150%, 40%, 20% and 0% (expressed as weight ratio % of leachate to waste; % g_{water} g_{waste}^{-1}). Inoculum was not used in order to evaluate the activity of the autochthonous biomass. All lab-scale bioreactors were air-tight sealed with screw caps (Omnifit C series) and were incubated at 35°C±1 in a thermostatic bath under static conditions (only manually shaken approx. once per week) over a period of 370 days. At the beginning of the experiments, anaerobic conditions were obtained adding nitrogen gas for approximately 15 minutes. All experiments were carried out in duplicate. BMP was also measured for leachate without MSWFF addition ("blank" reactor). The results of the methane production are reported as net values by subtracting the obtained blank value. The main characteristics of the leachate were: total solids (TS) of 1.4 (% wet weight); volatile solids (VS) 51.7 (% TS); total chemical oxygen demand (COD) of 2380 mg L^{-1}; pH of 8.4. Methane production was continuously monitored using a home-made wet-tip gasmeter connected to a data logger (Data Taker DT80). The CO_2 was removed from the biogas by an alkaline (NaOH 1M) trap. All gas data reported in this study referred to standard conditions.
Since the reactors with low moisture content produced a very small amount of methane, on experimental day 115 some leachate was added to the "20%" bottle in order to increase the leachate to waste ratio to 30 %.

7.2.3. Pilot-scale bioreactor

A pilot-scale reactor (Fig. 1) simulating an active landfill bioreactor (LBR) was used for the anaerobic degradation/stabilisation of the MSWFF in order to study the fate of the MSWFF in the landfill.

The reactor had a working volume of 1 m^3 and was filled with 280 kg of the MSWFF; moreover, since the results of the lab-scale reactors demonstrated that the MSWFF does not produce biogas if not rehydrated, the LBR was "activated" adding 460 L of leachate (same characteristics as reported in Section 2.2) and, thus, reaching a ratio of approx. 160% of leachate to MSWFF (weight). A gravel layer of approx. 20 cm (height) was placed on the bottom of the LBR in order to improve the leachate harvesting into the holding tank (1 m^3) located below the LBR.

The reactor was operated in batch mode (with no further waste or leachate addition) for 235 days under mesophilic condition (35±3°C). Reactor temperature was monitored and controlled by using a temperature probe (PT100) and a hot water jacket, respectively. Leachate was recirculated once a day in order to assure sufficient hydration of the MSWFF.

7.2.4. Samples characterisation and analytical methods

MSWFF total solids (TS, as %FF) and volatile solids (VS, as %TS), as leachate chemical oxygen demand (COD) and total volatile fatty acids (VFAs) were measured according to the Standard Methods (APHA, 2005). pH of the MSWFF was measured by UNI EN 12506, (2004).

The biological activity of the bio-dried MSWFF before and after bioreactor treatment was evaluated through BMP and respirometric tests for the determination of the anaerobic and aerobic stability indices, respectively.

Biogas composition was measured by gas chromatographic techniques as described elsewhere (Spagni et al., 2010).

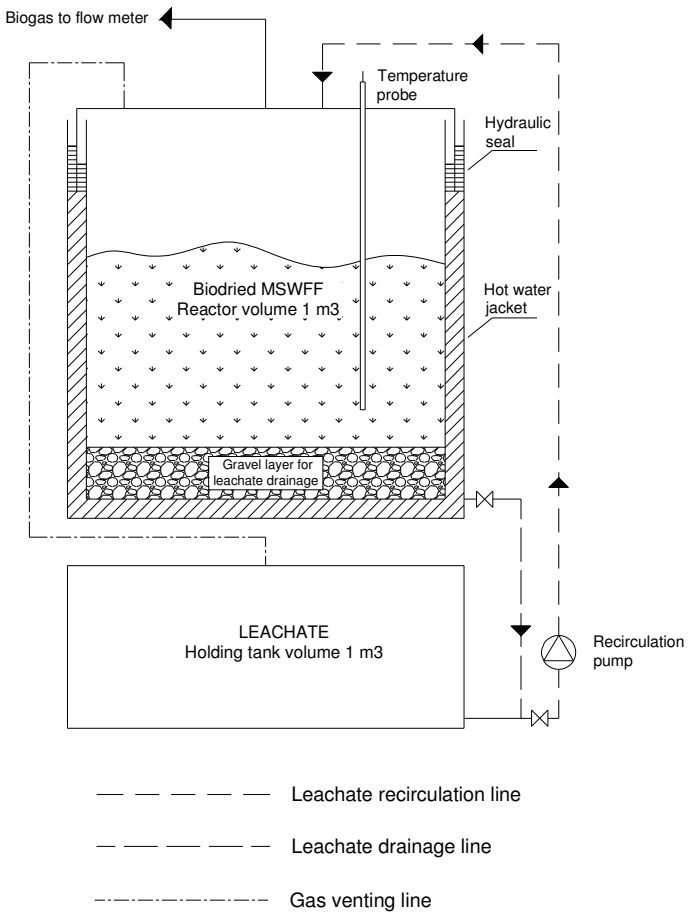

Fig. 1. Schematic of pilot scale LBR.

7.2.4.1. Aerobic stability indices

The aerobic stability was measured using the potential and real dynamic respirometric indices (PDRI and RDRI; $mgO_2\ kg_{TS}^{-1}\ h^{-1}$), and the cumulative oxygen demand observed in a period of 4 and 7 days (OD4 and OD7; $gO_2\ kg_{TS}^{-1}\ h^{-1}$).

The aerobic tests were performed by using an adiabatic respirometer (3022 Costech Instrument Cernusco S.N., Italy; Adani et al., 2004; 2006; UNI/TS, 2006) with minor modifications as reported in Grilli et al. (2009). Since the test duration affects the waste biodegradability (Binner and Zach, 1999; Wagland et al., 2009) and a lag-phase may occur after starting the respirometric test (Binner, 2003; Grilli et al., 2009), the samples were monitored for the respiration activity for at least 15 days.

The biodried material that formed the initial MSWFF was tested for RDRI (not re-hydrated) and PDRI adjusted to the optimal moisture content (75% of the water-holding capacity) by tap

water addition. On the contrary, since after the treatment in the pilot LBR the moisture content was higher that the water-holding capacity, the RDRI coincided with the PDRI.
The OD4 and OD7 were calculated on the data obtained during the measurements of the dynamics index defined as the area under the O_2 consumption rate curve over time (Binner and Zach, 1999; Grilli et al., 2009; Ragazzi et al., 2011).
Usually, the stability limits adopted in Europe refer to the aerobic respirometric units as the total content of organic matter (OM and estimated as VS); however, the heterogeneity of the tested MSWFF samples resulting from the plastic material content could introduce significant variability in the determination of the organic matter content. Therefore, as Ponsá et al., (2008) suggested, the units of the DRIs results were based on total solids content (TS %FF).

7.2.4.2. Anaerobic stability index

The material that resulted after 235 days of treatment in the LBR was tested for the anaerobic stability index using the BMP test (Owens and Chynoweth, 1993) with minor modification. The BMP tests were performed in duplicate by using 1-L Pyrex-glass bottles at constant temperature of 35±1 °C. Contrary to the anaerobic lab-scale reactors (see Section 2.2.), inoculum was added to BMP bottles applied to the resulted materials of the pilot-LBR in order to speed up the methanisation processes and so to reduce the test duration. Therefore, the bottles were filled with approx. 140 g of digested MSWFF sample (from LBR), 120 g of wet anaerobic granules from a full-scale UASB reactor (corresponding to about 20 g of TS), and sodium carbonate buffer (Na_2CO_3) and incubated for over 100 days.

7.3. Results and discussion

7.3.1. MSWFF characterisation

The solids content of the biodried MSWFF sample was 74.0±2.2 (% FF) and 50.3±11.3 (% TS) for TS and VS, respectively. The standard deviations (SD) of the TS and VS evidenced the high heterogeneous composition of the investigated sample, especially in the VS value that may be due to a significant content of large (sieved at 60 mm) pieces of non-biodegradable volatile matter (i.e. plastic).

Neutral pH (7.2±0.4) confirmed that the material is suitable for anaerobic digestion treatment in LBR.

The MSWFF density was 600 kg m^{-3} that is in agreement with other study on waste (Benbelkacem et al., 2010).

In spite of the high SD that is typically measured in MSW, Gonzalez-Ramirez et al. (2010) still suggest to estimate the organic matter (OM) content of the considered sample by the VS analysis.

The solids measured in the MSWFF agree or are slightly lower than data reported by other authors for other biodrying plants (Tambone et al., 2011); however, the data reported by Tambone et al. (2011) referred to biodried materials and not to the FF of the biodried MSW as used in this study.

7.3.2. Anaerobic lab-scale bioreactors

The trends of the cumulative methane production measured during the BMP tests on 200%, 150%, 40%, 20% and 0% bioreactors are reported in Fig. 2. Over the one-year test, the bioreactors have shown distinguished profiles in methane production in relation to the waste moisture content. The results confirm that the moisture content strongly affects the microbial activity and, thus, the methane production (Hernández-Berriel et al. 2008; Le Hyaric et al., 2012; Tambone et al., 2011). Moreover, the dried MSWFF (without leachate addition, 0% bottle) resulted stable under anaerobic conditions, since a negligible methanogenic activity was observed (Fig. 2) as other authors reported for the biodried MSW (Tambone et al., 2011). MSWFF can therefore be handled and landfilled due to its low water content, while their methanogenic potential can be easily reactivated by leachate addition in landfill bioreactors.

The maximum methane yield was obtained from the 150% bioreactor, with 69 Nm^3 CH_4 $tonFF^{-1}$ or 93 Nm^3 CH_4 $tonTS^{-1}$. Furthermore, the 150% sample also showed the highest methane production rate (6.09 Nm^3 CH_4 ton TS^{-1} d^{-1}) and the shortest lag phase (Fig. 2).

The BMP results also suggest that a positive effect on methanisation performance can be obtained by the water content of 40% and, although with even slower methanogenic activity, also by the 20% sample; these results also showed that the lower the moisture, the longer the lag phase.

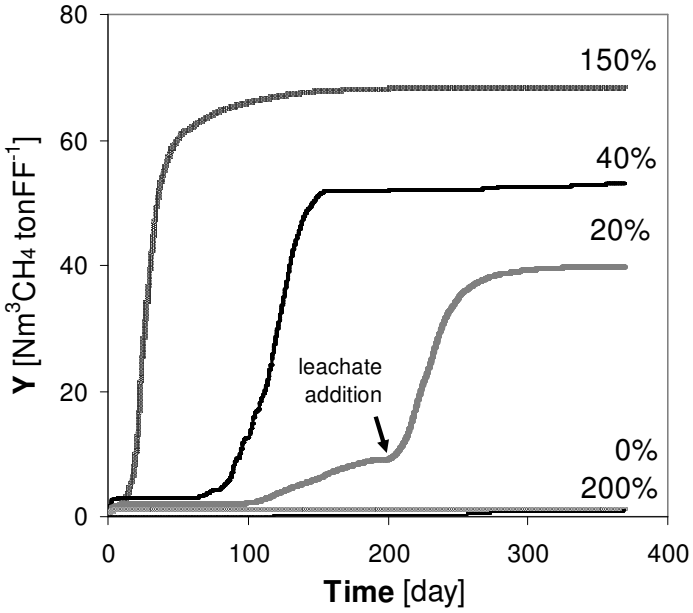

Fig. 2. BMP trends of the MSWFF at different moisture content in lab scale bioreactors.

Negligible BMP values were measured for both 200% and 0% reactors. The conditions of the 200% bioreactor led to the inhibition of the methanogenesis due to volatile fatty acids accumulation and thus to pH decrease to acidic conditions. In fact, at the end of the year of the study, when the two 200% bottles (duplicate samples) were opened chemical analysis on the produced leachate revealed a strong acidification to pH of 4.2 with total VFA concentration of approx. 70 g L^{-1}.

On the contrary, the 0% BMP test indicated that the material obtained by the bio-drying process does not support methanogenic activity because of the very low MSWFF moisture content (Hernandez-Berriel et al., 2008; Tambone et al., 2011). Thus, the MSWFF remains stable when landfilled until leachate is added by recirculation (landfill bioreactor).

The 20% reactor showed a longer lag phase and produced less methane than the 150% and 40% sample bottles. Since it also reached the plateau in methane yield (Fig. 2), leachate was re-added (see Section 2.2) in order to evaluate if the increase of the moisture content can support microbial methanogenic activity and thus can improve MSWFF biostabilisation. This further leachate addition aimed to simulate the leachate recirculation in the landfill bioreactor. Fig. 2 shows that the moisture increase in the 20% reactor to approx. 30% (% g_{water} g_{waste}^{-1}) led to a significant improvement in methane yield from 12 to 54 Nm^3 CH_4 $tonTS^{-1}$. Therefore,

managing the leachate recirculation in landfill bioreactors and, thus, maintaining the moisture content above a threshold limit (30% in this study) can optimise the MSWFF biostabilisation. This behaviour also confirms that the biodrying treatment only partially stabilises the waste by reducing the humidity content of the organic matter (Tambone et al., 2011).

The "blank" reactors (containing only leachate) produced negligible amount of methane, and thus, the relatively high amount of organic matter (COD=2380 mg L^{-1}) of the leachate resulted refractory.

Contrary to the methane production, the moisture content seemed to not significantly affect the biogas composition; in fact, biogas methane concentration varied between 50-60% with CO_2 as the remaining fraction.

Microbial activity is highly influenced by moisture content. For instance, Hernández-Berriel et al. (2008) reported that the highest methane production rates occur at waste moisture of 60–80%. The results confirms that the moisture content affects both the methane production rate and yield using MSWFF as substrate (Barlaz et el., 1990; Benbelkacem et al., 2010; Bogner, 1990; Le Hyaric et al., 2012; Pommier et al., 2007). It is noteworthy that our results confirm that the lower methane yields that were observed with lower moisture content, seems to be mainly observed with long-term experiments (more than one year). Hence, prolonging the experimental duration may lead to moisture decrease due to biochemical reactions or water vapour lost with the biogas. In fact, Hernandez-Berriel et al. (2008) reported the decrease of the waste moisture content during anaerobic batch methanisation assays.

7.3.3. Methane production in pilot LBR

The pilot-plant was started-up with 280 kg of biodried MSWFF re-hydrated by leachate addition, in order to increase the moisture content from 26 to 72 %FF and thus to attain the weight ratio of around 150% ($g_{water}\ g_{waste}^{-1}$), according to the results obtained with the lab-scale tests.

The pilot LBR showed a high methane productivity up 1.1 $Nm^3\ CH_4\ d^{-1}$, resulting in a maximum specific methane production up to 5.9 $Nm^3\ CH_4\ ton\ TS^{-1}\ d^{-1}$. The pilot-scale biomethanisation experiment confirmed the results obtained from the lab-scale experiments; in fact, a significant microbial methanisation can be achieved from the re-hydrated MSWFF that led to waste biostabilisation in approximately 200 days (Fig. 3).

With the LBR specific methane production, Fig. 3 also shows the CH_4 production from the lab-scale anaerobic reactor with leachate addition of 150% (g_{water} g_{waste}^{-1}). It is noteworthy that the methane yield of the pilot-scale LBR (98 Nm^3 CH_4 ton TS^{-1}) strongly agrees with the data obtained at lab-scale (93 Nm^3 CH_4 ton TS^{-1}). The specific methanogenic production rate of the lab-scale reactor (6.1 Nm^3 CH_4 ton TS^{-1} d^{-1}) also agrees with the data obtained at pilot scale (5.9 Nm^3 CH_4 ton TS^{-1} d^{-1}).

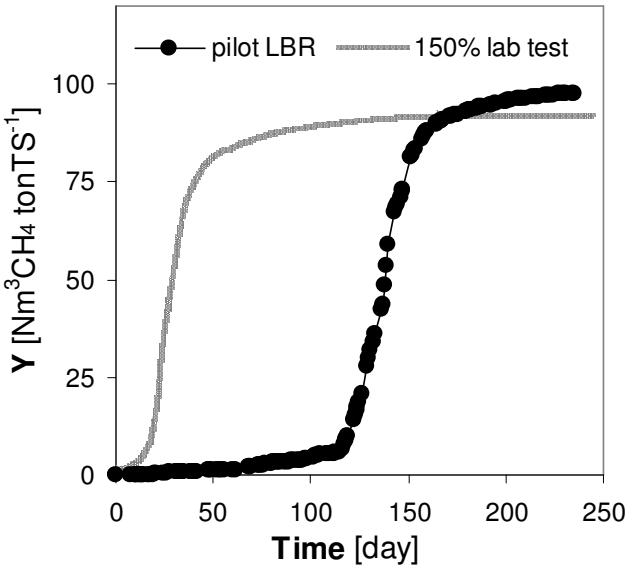

Fig. 3. Methane production trends of the MSWFF in pilot LBR and 150% lab scale bioreactor.

The two reactors (lab- and pilot-), however, showed very different lag-phase that is probably due to the difficulty in the homogenisation of large amount of MSWFF and leachate used in the pilot LBR experiment. This result, obtained at the pilot-scale, highlights the much longer time necessary to start-up a full-scale landfill bioreactor treating biodried MSW.

The test ended on day 235 when the methane production rate reached values below 25 NL CH_4 ton TS^{-1} d^{-1}.

Moisture content measured at the end (63%) of the experiments was slightly lower than that measured at the beginning (72%). Therefore, as already stated by other authors (Hernandez-Berriel et al. 2008), moisture content decreases during the batch anaerobic degradation of MSW. Moreover, a decrease of the VS content (of approx. 8%) of the MSWFF was observed

as also reported in other studies using MSW (e.g. Hernandez-Berriel et al. 2008). The standard deviation of VS measurements on the anaerobically digested MSWFF in LBR significantly decreased from 11.3 to 7.3 %TS probably due to a homogenisation effect.

7.3.4. Biostabilisation due to LBR treatment

The MSWFF biostabilisation due to LBR treatment was evaluated by aerobic and anaerobic indices. MSWFF was tested for RDRI and PDRI while for the digested MSWFF in the pilot LBR the RDRI coincided with the PDRI.

The data trends of PDRI, OD 4 and OD7 obtained during the aerobic biostability test performed on the biodried sample (MSWFF) are reported in Figure 4 as an example.

The DRI tests showed values of less than 200 and 1525 gO_2 $tonTS^{-1}$ h^{-1} for RDRI and PDRI, respectively (Table 1). The results demonstrated that MSWFF was apparently stable, in agreement with data reported in Tambone et al. (2011), who also observed significant difference between the RDRI and PDRI of biodried MSW. Thus, based on RDRI, the MSWFF showed a high biological stability owing to low moisture content that hinders the microbiological activity.

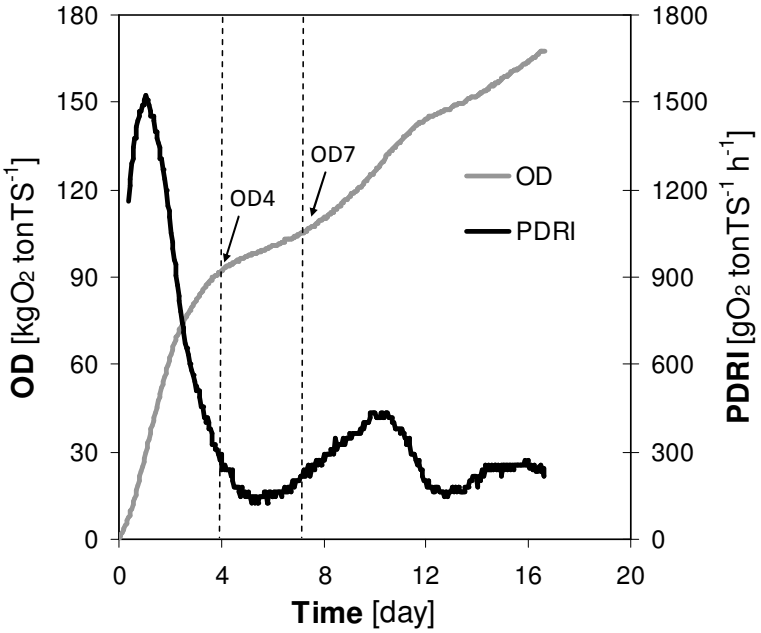

Fig. 4. PDRI test data measured for the MSWFF.

On the contrary, PDRI (after rehydration) revealed that MSWFF was not stable and, indeed, a high aerobic microbial activity was observed. The measured PDRI resulting higher than the proposed threshold values of 500-700 gO_2 $tonTS^{-1}$ h^{-1} (Scaglia et al., 2010) for biotreated MSW, cannot be considered biologically stable. The PDRI obtained for the rehydrated MSWFF is also in agreement with the results obtained on raw (untreated) MSW (Adani et al., 2004), confirming once more their instability.

The OD trends confirmed the results obtained using RDRI and PDRI (Fig. 5). Note that, in Figure 5, the RDRI MSWFF curve is much more similar to R/PDRI LBR than to PDRI MSWFF. Therefore, the results of the aerobic indices (Table 1) confirmed, once more, that the MSWFF was apparently stable because of the low moisture content.

The R/PDRI measured on the MSWFF after the biostabilisation by the anaerobic treatment in LBR, demonstrated a significant improvement of the aerobic biostability index. In fact, the LBR treatment decreased ten times the PDRI of the digested MSWFF ($127gO_2$ $tonTS^{-1}$ h^{-1}) if compared to the initial material. Therefore, the treatment in landfill bioreactors, if properly managed, can be considered as an effective biostabilisation process for MSWFF.

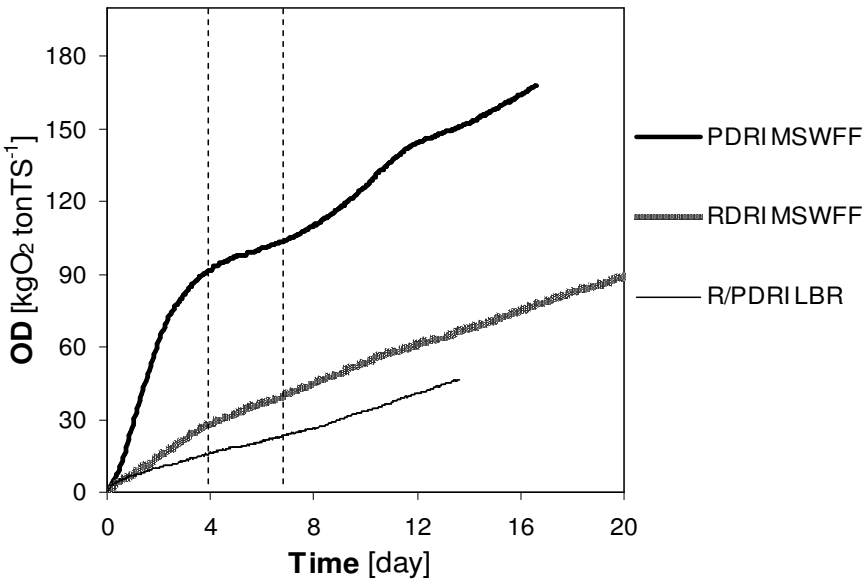

Fig. 5. OD4 and OD7 trends of the MSWFF before and after pilot LBR treatment. Dashed lines refer to OD4 and OD7.

The anaerobic stability index showed a significant improvement (74% on TS) of the biostability of MSWFF after LBR treatment (Table 1). As for the aerobic respiration, MSWFF showed significant methanogenic activity after moisture recovery. The values reported in table 1 for the BMP test for rehydrated MSWFF are typical of untreated MSW (Ponsá et al., 2008).

The results of the anaerobic index reported are in agreement with those obtained under aerobic conditions (Table 1). All anaerobic and aerobic indices decreased more than 74% (Table 1), and the highest values measured for the PDRI (92%) and OD4 (82%) suggested a considerable stabilisation mainly due to the degradation of the easily biodegradable organic matter; furthermore, the similar values for the stability increase of OD7 (78 %) and BMP (74%) agree with the relationship between the aerobic and anaerobic indices reported in literature (Binner and Zach, 1999; Cossu and Raga, 2008; Grilli et al., 2009; Ponsà et al., 2008).

Table 1. Anaerobic and aerobic stability indices of the MSWFF before and after pilot LBR treatment.

MSWFF	**BMP**		**R/PDRI**	**OD4**	**OD7**
	[Nm^3CH_4 $tonFF^{-1}$]	[Nm^3CH_4 $tonTS^{-1}$]	[gO_2 $tonTS^{-1}h^{-1}$]	[kgO_2 $tonTS^{-1}$]	
before rehydration	-	-	<200	29	41
after rehydration	72	98	1525	92	105
after LBR treatment	9	25	127	17	23
stability increase (%)	87%	74%	92%	82%	78%

7.4. Conclusions

Biodried MSWFF was apparently stable due to low moisture content that slows down the microbial activity. The lab-scale anaerobic bioreactors demonstrated that a proper moisture content lead to a complete biodegradation of the organic matter contained in the biodried MSWFF.

Using a pilot-scale LBR, MSWFF stabilisation was achieved, suggesting that the leachate recirculation could be an effective approach to accomplish the anaerobic biodegradation and biostabilisation of biodried MSWFF after landfilling.

The biostabilisation of the material resulting from the LBR treatment was confirmed using anaerobic and aerobic stability indices. All anaerobic and aerobic indices showed the stability increase of approximately 80%. The similar values for the stability increase of OD7 and BMP well agrees with the relationship between the aerobic and anaerobic indices reported in literature.

Acknowledgements

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Chapter 8

EFFECT OF NITRATE AND NITRITE ADDITION ON LEACHATE CHARACTERISTICS IN A SIMULATED LANDFILL BIOREACTOR*

Abstract

The aim of this study was to evaluate the effect of the addition of nitrate and nitrite on the leachate characteristics of old landfills. Attention was paid on the nitrogen cycle in order to evaluate if the Anammox process could take place into the system. The results confirm that nitrate and nitrite addition has a significant effect on the leachate characteristics in landfills operated as bioreactor. In particular, the recycle of leachate generated in old landfill through well-stabilised wastes caused the removal of both COD and ammonia. Ammonia concentration decreased continuously for the entire duration of the experiment. On the contrary, COD removal ceased after approximately 100 days of experimentation due to the exhaustion of the biodegradable organic matter. Although the constant COD values, the bioreactor was able to sustain denitrification processes. It was noteworthy that ammonia removal continued after nitrite addition in spite of the stable COD. These results, therefore, sustain the hypothesis that the Anammox process could take place in old landfills if properly managed.

Key words: Landfill bioreactor, Nitrogen removal, Anammox

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8.1. Introduction

Landfill leachate treatment is usually accomplished by multistage systems using chemical, physical and biological processes. Biological processes have been proved to be effective in leachate treatment, especially for organics and nitrogen removal. Biological nitrogen removal is accomplished by the nitrification and denitrification processes (Lema et al., 1988; Renou et al., 2008). During landfill aging, the concentration of organic compounds into leachate normally decreases and becomes less biodegradable, whereas ammonia concentration tends to increase. Therefore, the leachate generated in old landfills results in a high-strength wastewater characterized by a low BOD/TKN ratio (Lema et al., 1988; Kjeldsen et al., 2002). Therefore the denitrification process can only be achieved if external biodegradable organic matter is added (Manoharan et al., 1989; Spagni et al., 2008; 2009). Moreover, the very high ammonia and total Kjeldahl nitrogen (TKN) concentrations usually present in the "old" landfill require very high oxygen demand that greatly increases the operational costs.

Over the last decades, the landfills have been operated as bioreactor where the produced leachate is recirculated inside the landfill. Although significant benefits associated with landfill bioreactors have been demonstrated, ammonia concentrations tend to be higher than those present in leachate generated in conventional landfills (Kjeldsen et al., 2002; Berge et al., 2005).

Biological nitrogen removal from sanitary landfill leachate is usually accomplished by ex-situ systems (Robinson and Olufsen, 2007; Robinson et al., 2009). However, in recent years, there has been a great interest in using the landfill as bioreactor also for nitrogen removal: in this case, the landfill is operated as aerobic and anaerobic bioreactor where both nitrification and denitrification processes can take place (Onay and Pohland, 1998; Berge and Reinhart, 2003; Berge et al., 2005). Another possibility for nitrogen removal is via ex-situ ammonia oxidation followed by in-situ nitrate denitrification: in this case the produced leachate is nitrified before being recirculated so to use the landfill itself for anoxic nitrate reduction (Jokela et al., 2002; He et al., 2006).

Biological nitrogen removal is achieved by nitrification and denitrification processes. Over the last decades there has been a growing interest in using nitrite as shortcut for nitrification (nitritation, ammonia oxidation to nitrite) and denitrification (denitritation, nitrite reduction to nitrogen gas) processes. Indeed, the main advantages of the nitrite

pathway are the decrease in oxygen consumption and the reduction of organic matter demand (Lai et al., 2004; Peng et al., 2008; Turk and Mavinic, 1989).
The aim of this study was to evaluate the effect of the addition of nitrate and nitrite on the leachate characteristics of old landfills. Attention was paid on nitrogen cycle in order to evaluate if the Anammox process could take place into the system.

8.2. Materials and methods

Reactor set-up

The study was carried out using a reactor (lysimeter) of 2 m height and 20 cm diameter (approx 60 L). In addition a tank of 50 L was placed below the reactor in order to collect the landfill leachate (Fig. 1). The reactor and the tank were filled with municipal wastes (26.5 kg wet weight) and leachate (41 L) collected in a portion of a closed municipal landfill (sited in the province of Pavia, Northern Italy) in order to use wastes and leachate that can be defined as "stable". The reactor was maintained at 35±1 °C. The leachate was recirculated through the wastes one or twice per day using a peristaltic pump. A second peristaltic pump was used for nitrate or nitrite addition. A wet-tip gas meter was used for biogas measurement.

Analytical methods

The leachate characteristics were monitored for pH, total suspended solids (TSS), volatile suspended solids (VSS), ammonia, nitrite, nitrate, total Kjeldahl nitrogen (TKN), total chemical oxygen demand (CODt), filtered COD (CODf), alkalinity to pH 4.3 (Alk4.3) and conductivity at 20°C (K20) according to the Standard Methods (APHA, 2005). Biogas was measured by gaschromatographic techniques. Sample filtration was carried out using Whatman GF/C filters.

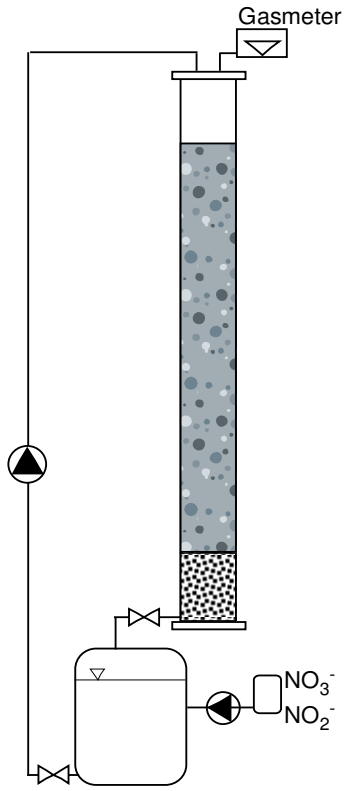

Figure 1. Schematic diagram of the simulated landfill bioreactor.

8.3. Results and discussion

The results confirm that the leachate recycle through the wastes has a significant effect on its characteristics. The COD concentrations decreased almost continuously for approximately 100 days (Fig. 2). The decrease in COD concentration confirms that both wastes and leachate were collected in cell that was not in operation since a long time (a few years) and, therefore, can be classified as stable. In particular, COD decreased very rapidly from approximately 4700 to 3200 mg/L during the first 10-20 days and then continued to decrease to 2000 mg/L. The very high COD decrease during the first 10-20 days can be explained by the removal of the small biodegradable fraction of the organic matter still present in the leachate or by filtration (so removal of the particulate COD) passing through the waste or by absorption on the waste. After approximately 100 days, the COD concentration remained almost stable confirming very low biological activity of the wastes.

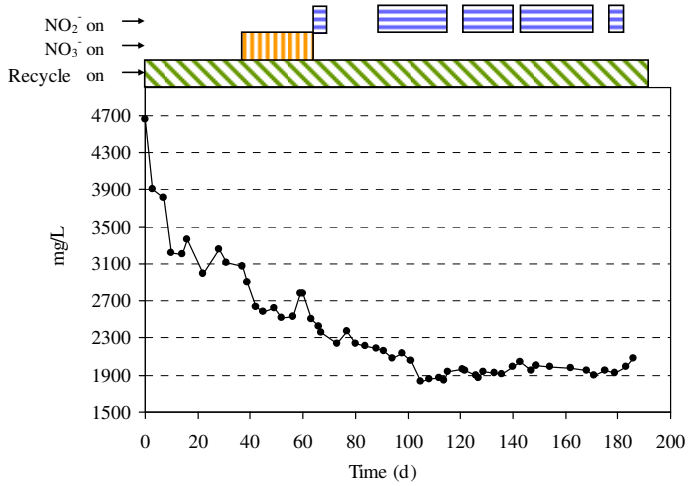

Figure 2. COD concentration of the landfill leachate. Above the graph the time when the recycle, the nitrate and the nitrite addition was performed.

In the same way to COD, ammonia concentration also decreased very rapidly during the first 10-20 days suggesting that chemico-physical processes (such as absorption) could prevail during the first leachate passage through the waste (Fig. 3). In particular, ammonia concentration decreased from approximately 1100 to 500 mg N/L in 10-20 days and, thereafter, decreased continuously till 200-300 mgN/L in approximately 170-180 days. It is of note that ammonia removal continued continuously although COD concentration remained almost constant after 100 days of experimentation. Therefore COD removal can be affected by different biological or chemico-physical processes than ammonia removal.

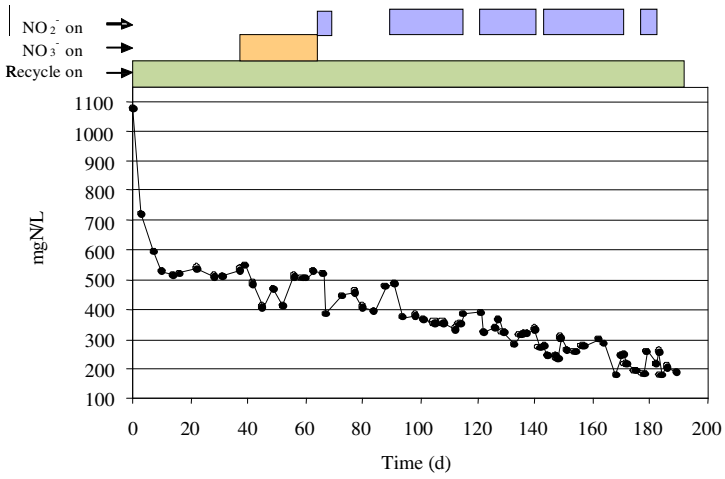

Figure 3. Ammonia concentration of the landfill leachate. Above the graph the time when the recycle, the nitrate and the nitrite addition was performed.

After approximately 40 days with leachate recycle, nitrate was added in order to evaluate if denitrification processes can take place in the wastes. Figure 4 confirms that nitrate can sustain the biological denitrification processes with COD removal. With the end of the nitrate addition its concentration remained rather low (below 2 mgN/L).

It is noteworthy that biogas production greatly increased with nitrate addition (Fig. 5). In fact, biogas production was very low during the first days of operation confirming the low biodegradability of the organic matter still present into the leachate and wastes. With the addition of nitrate, biogas production increased from 0.2-0.5 to 5-10 L/d: the biogas composition also changed from methane and carbon dioxide (20-35:65-80%) to nitrogen and carbon dioxide (35-45:55-65%). This seems to indicate that biological denitrification processes are more significant than the previous methane production ones.

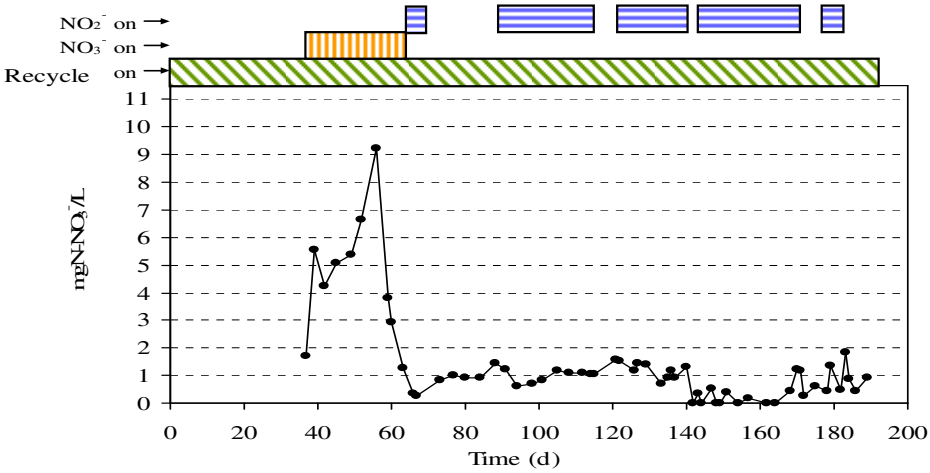

Figure 4. Nitrate concentration of the landfill leachate. Above the graph the time when the recycle, the nitrate and the nitrite addition was performed.

Because the main objective of the study was the evaluation if the Anammox processes can take place in the waste with leachate recirculation, after approximately two months of operation nitrite was added to the leachate. Nitrite addition was switched off when the profiles of nitrite concentration increased (Fig. 6).

Nitrite concentration is the electron acceptor for the Anammox microorganisms as in the following equation:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O$$

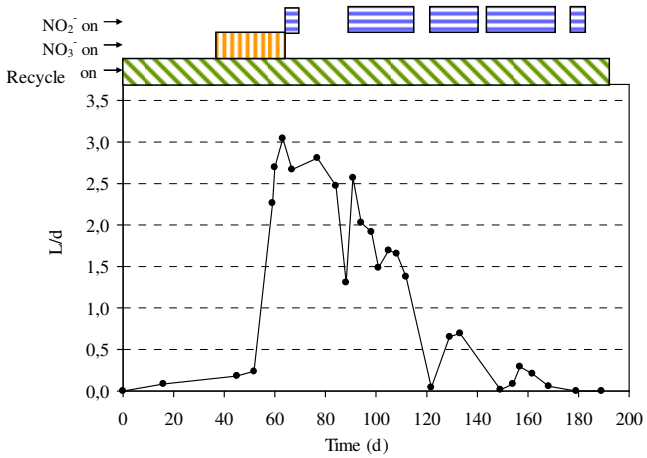

Figure 5. Biogas production from the landfill bioreactor. Above the graph the time when the recycle, the nitrate and the nitrite addition was performed.

Therefore ammonia can be removed without air addition and biodegradable organic matter. Figure 6 shows that the added nitrite to the leachate was denitrified. In is noteworthy that during the first 40 days of nitrite addition, the nitrite removal corresponded to the COD removal: on the contrary, after, 100 days of experimentation, COD removal ceased whereas nitrite as ammonia removal continued. Therefore, the results suggest that the ammonia removal could be sustained via nitrite removal by the Anammox process.

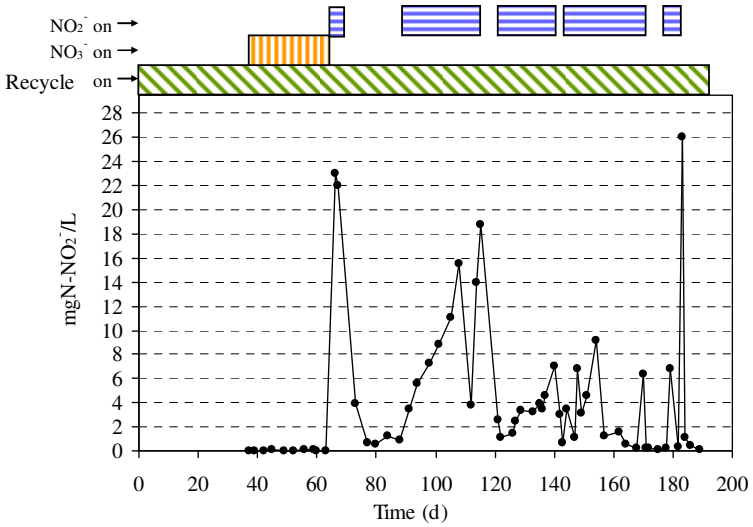

Figure 6. Nitrite concentration of the landfill leachate. Above the graph the time when the recycle, the nitrate and the nitrite addition was performed.

8.4. Conclusions

The results confirm that nitrate and nitrite addition has a significant effect on the leachate characteristics in landfills operated as bioreactor. In particular, the recycle of leachate generated in old landfill through stabilised wastes caused the decrease of the measured parameters (COD, and ammonia).

Ammonia concentration decreased continuously for the entire duration of the experiment. On the contrary, COD removal ceased after approximately 100 days of experimentation due to the exhaustion of the biodegradable organic matter. Although the constant COD values, the bioreactor was able to sustain denitrification processes. It was noteworthy that ammonia removal continued after nitrite addition in spite of the stable COD. These results, therefore, sustain the hypothesis that the Anammox process could take place in old landfills if properly managed.

Acknowledgment

This study has been partly supported by the MIUR-PNR-FIRB project “Risparmio energetico con valorizzazione dei bacini secondari di energia quale fonte energetica distribuita”, unit Natural Energy from Waste (NEW).

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Chapter 9

INNOVATIVE TWO-STAGE ANAEROBIC PROCESS FOR EFFECTIVE CODIGESTION OF CHEESE WHEY AND CATTLE MANURE*

Abstract

The valorisation of agroindustrial waste through anaerobic digestion represents a significant opportunity for refuse treatment and renewable energy production. This study aimed to improve the codigestion of cheese whey (CW) and cattle manure (CM) by an innovative two-stage process, based on concentric acidogenic and methanogenic phases, designed for enhancing performance and reducing footprint. The optimum CW to CM ratio was evaluated under batch conditions. Thereafter, codigestion was implemented under continuous-flow conditions comparing one- and two-stage processes. The results demonstrated that the addition of CM in codigestion with CW greatly improved the anaerobic process. The highest methane yield was obtained co-treating the two substrates at equal ratio by using the innovative two-stage process.

The proposed system reached the maximum value of 258 mL $_{CH4}$ g_{VS}^{-1}, which was more than twice the value obtained by the one-stage process and 10 % higher than the value obtained by the two-stage one.

Keywords: Codigestion; Cheese whey; Cattle manure; Biogas; Two-stage process.

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9.1. Introduction

The proper management and valorisation of agroindustrial waste (i.e. organic waste) through anaerobic processes represents a significant opportunity to combine waste treatment and renewable energy production (Esposito et al., 2012).

CW is the main by-product of the dairy industry. It is characterised by a very high organic load and low buffer capacity; consequently, the direct anaerobic treatment of raw whey can lead to rapid acidification which results in low biogas productivity (Ghaly, 1996; Malaspina et al., 1996; Saddoud et al., 2007). Contrary to CW, CM is characterised by low C/N ratio (Esposito et al. 2012). Owing to the high nitrogen content of CM, ammonia tends to accumulate in digesters resulting in anaerobic digestion (AD) microbial processes inhibition (Nielsen and Angelidaki, 2008). Several studies have demonstrated that the codigestion of CW with CM can maintain favourable pH and improve biogas production (Gelegenis et al., 2007; Ghaly, 1996) but the optimal co-substrates ratio and the theoretical gas yield greatly vary according to each specific case (Esposito et al., 2012; Saddoud et al., 2007).

AD is not widespread in the dairy industry because CW normally displays high acidification potential and requires long hydraulic retention times (HRTs), and because of the small scale and fragmentation of dairy factories. Over the last decade, various bioreactor configurations have been evaluated and proposed for biogas improvement from organic waste (Nasir et al., 2012; Nizami and Murphy, 2010). Igoni et al. (2008) stated that simple and reduced design systems suffer less frequently from technical failures, and this results in economic benefits because of the reduced costs in process design, construction and management. On the contrary, the codigestion and two-stage reactors can provide higher treatment efficiency and process stability in relation to the single-substrate digestion and the use of one-stage processes (Nasir et al., 2012; Saddoud et al., 2007).

This study aimed to develop an innovative two-stage process devoted to the co-digestion of CW and CM, which could combine the advantages of the simplicity of conventional processes with the high efficiency of multistep reactors. To this end, a simple continuous two-stage process was designed and developed, so that the first acidogenic stage was directly inserted into the methanogenic vessel with a concentric design (Fig. 1) in order to reduce the footprint. The latter was compared with conventional one- and two-stage processes (Fig. 1). To the best of the authors' knowledge, this is the first study that aims to improve the codigestion of CW and CM by evaluating different design configurations.

9.2. Methods

9.2.1. Substrates and inocula

The agro-zootechnical wastes used as substrate (S) sources for the anaerobic codigestion experiments were cheese whey (S_{CW}) and cattle manure (S_{CM}), obtained from a dairy factory and a cattle farm, respectively, both located in the Emilia-Romagna Region (Northern Italy). Since CM was provided in a semi-solid state, before being used, it was diluted with tap water (1 manure: 2 water, v/v) and sieved (53-μm opening).

Three different inocula were tested as seed for the anaerobic digestion experiments. They consisted of methanogenic consortia from (a) an olive mill (I_{OM}) wastewater collected from an olive mill located in the Liguria Region (Northern Italy), (b) manure from the same cattle farm (I_{CM}) cited above and (c) sludge from a bench-scale reactor (I_R) treating organic fraction municipal solid waste as described in Bertin et al. (2012). The main characteristics of the substrates and inocula are reported in Table 1.

9.2.2. Batch tests

Batch tests were carried out as preliminary investigation to identify the optimal operating conditions for the anaerobic codigestion to be applied in the continuous reactors.

The methanogenic activity of the single wastes and in codigestion was measured by the biochemical methane potential (BMP; Owen et al., 1979) with minor modifications as described in Bertin et al. (2012). The tests were conducted in triplicate in 100 mL Pyrex-glass bottles started-up by adding 5 mL of inoculum and 50 mL of substrate consisting of the codigestion waste mixtures. The bottles were incubated at 35±0.5°C. The monitoring was carried out until complete methane production depletion (up to 100 d).

Three series of BMP tests were performed. The first series was conducted to evaluate the activity of the three inocula (I_{OM}, I_{CM} and I_R) on the two substrates (S_{CW} and S_{CM}) tested in codigestion (in equal volume ratios). The second BMP test series was conducted to identify the optimal mix ratio of the two substrates using I_R as inoculum; therefore, the two substrates were digested at different S_{CW}:S_{CM} ratios ranging from 0:100 % v/v at progressive variations of 10%.

The third set of batch tests aimed to evaluate the optimal acidogenic stage conditions to start up the two-stage process. The experiments were performed for the short-term biochemical hydrogen potential (BHP) test as described by Giordano et al. (2011) with minor modifications. Contrary to the BMP tests, the pH was initially adjusted to 6.0±0.5 by diluted HCl to improve the acidogenesis. The tests lasted for two weeks and ceased when methane

was observed in the biogas. Moreover, contrary to Giordano et al. (2011), the inoculum (I_R) was not subjected to any "hydrogen-production" pretreatment. The reactors were fed with 50:50-S_{CW}:S_{CM} volumetric ratio. The acidogenesis stage was evaluated at room temperature (approximately 20°C, i.e. without temperature control) and mesophilic (35°C) conditions.
The batch tests were monitored daily during the first 4-6 experimental days and weekly afterwards.

9.2.3. Continuous codigestion experiments

The experiments were carried out using three bench-scale reactors, where one- and two-stage processes were set-up. The one-stage process was studied in a completely mixed reactor (R1, Fig. 1a). The two-stage process was investigated using two different designs: in the first design (R2), a second smaller completely mixed reactor was added before the methanogenic vessel (Fig. 1b). The second two-stage reactor (R3) consisted of a single container, which included both the acidogenic and the methanogenic stages. The former was concentrically integrated into the latter, so that the acidified effluent was fed by gravity into the methanogenic phase (Fig. 1c).

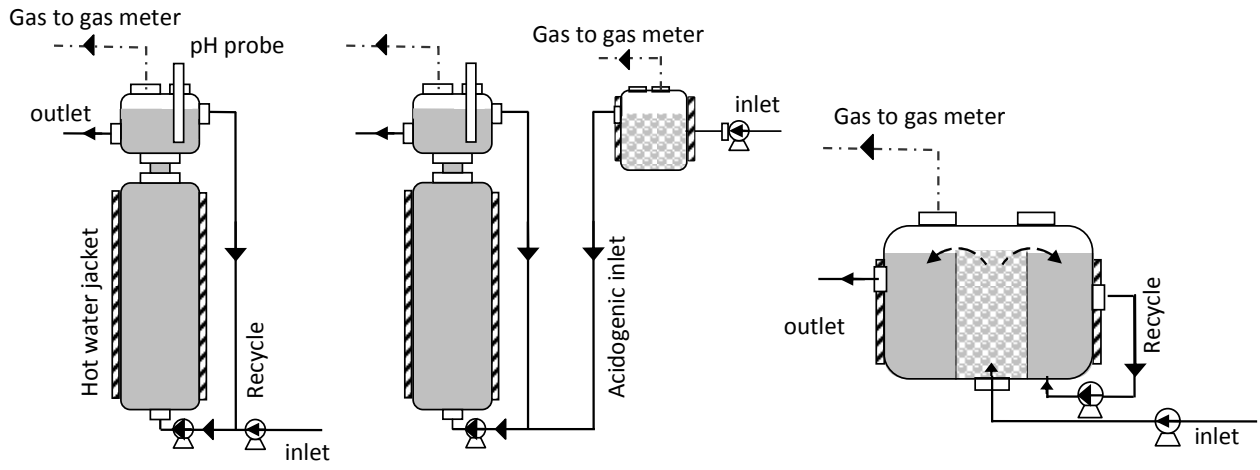

Figure 1. Schematic diagrams of the three reactors: a) one-stage reactor (R1); b) two-stage reactor (R2); c) two-stage concentric reactor (R3). (Not drawn to scale)

The methanogenic reactors had working volumes of 500 mL for R1 and R2, and 790 mL for R3; the acidogenic phase had a working volume of 120 and 190 mL in the R2 and R3, respectively. The three reactors were fed using peristaltic pumps set to ensure hydraulic

retention times (HRTs) of 20 d for the methanogenic phase and of 5 d for the acidogenic phases, resulting in organic loading rates (OLRs) of 1.8 and 1.7 kg_{COD} m^{-3} d^{-1} for the one- and two-stage reactor, respectively. On the basis of the results obtained by batch tests (see Section 3.2), the reactors were inoculated by using anaerobic sludge I_R and fed by the substrate ratio of 50:50 (v/v S_{CM}:S_{CW}). The bench-scale plants were maintained at 35±1°C.

Before starting the experiments, the reactors were operated for approximately one month in order to acclimate the biomass to the substrate. The reactors were operated for more than two months and their performance was evaluated under (almost) steady-state conditions, assumed as performance variations (in terms of COD removal rate) of less than 15 %.

9.2.4. Analytical methods

Total solids (TS), volatile solids (VS) and soluble chemical oxygen demand (sCOD) were measured according to standard methods (APHA, 2005). Carbohydrates were estimated according to Dubois et al. (1956) and proteins were estimated using the Bio-Rad Protein Assay.

The biogas produced by the three reactors was measured by home-made gas-meters. Biogas composition, volatile fatty acids (VFAs) and pH were measured as described in Bertin et al. (2012).

9.3. Results and discussion

9.3.1. Substrates characterisation

S_{CW} and S_{CM} were different mainly due to their content of organic matter and pH (Table 1). In fact, S_{CW} had higher concentrations of carbohydrates and proteins than S_{CM} (Table 1). On the contrary, S_{CM} had a pH that was significantly higher than that of S_{CW} (Table 1). Therefore, as also proposed by other authors (Dareioti et al., 2009; Gelegenis et al., 2007; Kavacik and Topaloglu, 2010), the addition of CM to CW in codigestion can result in more robust and effective AD (Esposito et al., 2012).

9.3.2. Batch tests

The first set of batch tests was performed to evaluate three different inocula. The BMP experiments resulted in methane yields of 26±4, 257±5 and 320±9 L $_{CH4}$ kg $_{VS}^{-1}$ for I_{OM}, I_{CM} and I_R, respectively. The average methane concentration in the biogas generated by I_R was

also higher (68±7 %) than that observed in the biogas generated by I_{OM} (64±4 %) and I_{CM} (58±5 %). Thus, I_R was used for the codigestion of S_{CM} and S_{CW} since it presented remarkably higher methane production.

The second set of BMP tests was conducted in order to evaluate the impact of different S_{CW}:S_{CM} ratios on AD mediated by I_R. The methane yields obtained using S_{CW} and S_{CM} separately were 12±3 and 131±7 mL $_{CH4}$ g $_{VS}^{-1}$, respectively. The BMP test using S_{CW} (S_{CW}:S_{CM}-100:0) surprisingly showed very low methane production. However, chemical analyses demonstrated an accumulation of VFAs (data not shown) with pH decrease (down to 4.2) just a few days after the beginning of the test. Similar findings were reported by other authors (Ghaly, 1996; Malaspina et al., 1996) who observed acidification and, thus, methanisation inhibition during AD of CW. The combination of S_{CW} and S_{CM} resulted in higher methanogenic performances (Fig. 2). In fact, methane yield of the codigestion (S_{CW}:S_{CM}-50:50) improved to 320±9 mL_{CH4} g_{VS}^{-1} that is 2.5 the value obtained by CM and 27 times the value obtained by CW when used alone.

Although the methane yield increased with S_{CW} (S_{CW}:S_{CM} ratios of 0:100 to 50:50), CH_4 production fell when the S_{CW} fraction was higher than 60 % (Fig. 2). Therefore, the results demonstrate that codigestion seems much more robust with the increase of the S_{CM} fraction and there is a threshold below which the process tends to acidify the medium. In fact, acidification to pH values below 6.2 (value for S_{CW}:S_{CM} 70:30) was observed when the S_{CW} fraction was higher than 60 %. On the contrary, the increase of the S_{CW} fraction from 0 to 60% greatly improved the methane yield as a result of the higher content of biodegradable organic matter of S_{CW} as also proposed by Kavacik and Topaloglu (2010).

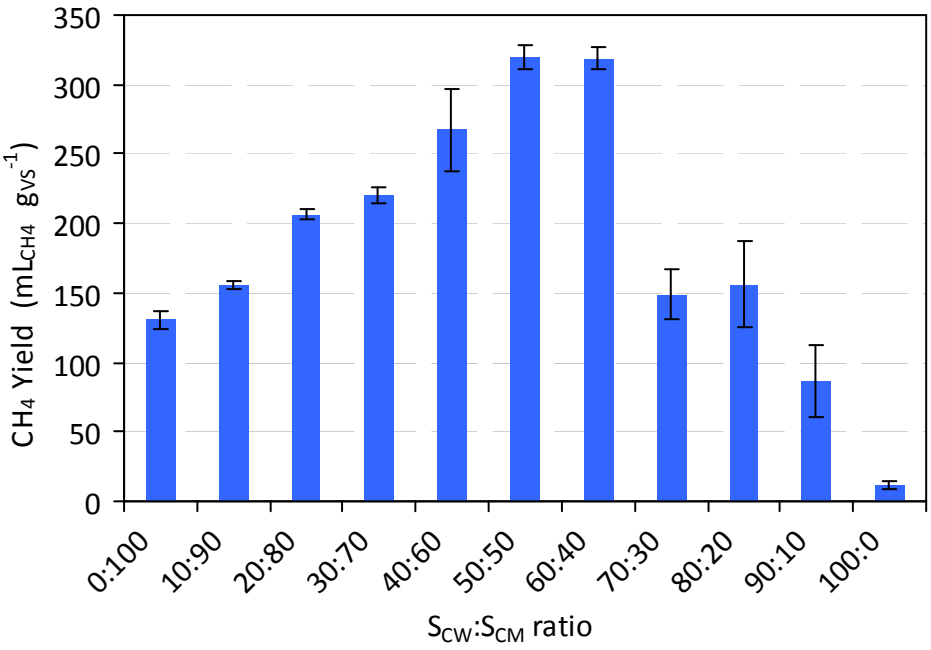

Figure 2. Methane yields measured at different cheese whey: cattle manure volume ratio (SCW:SCM). Bars for SD.

Methane concentrations comprised between 54 and 66 % were measured in the headspaces irrespective of the applied S_{CW}:S_{CM} ratios, demonstrating the low effect of the tested feed on biogas composition.

Maximum CH_4 production rate and complete CH_4 production depletion were measured after approximately 20 and 50 experimental days, respectively. Therefore, the bench-scale methanogenic stages were designed for HRT 20 d.

The third batch experiments were carried out to determine the optimal conditions of the acidogenic phase of the two-stage codigestion. The pH of the anaerobic liquor decreased just the day after the beginning of the trials by reaching values of 4.5-5.0 at the end of the experiments. The acidification, due to accumulation of VFAs, caused the inhibition of methanogenic activity coupled with hydrogen accumulation in the biogas (Chen et al., 2008). Total accumulation of VFAs up to 3,700 mg L^{-1} was observed in the acidogenic reactors. The main VFAs produced were acetic (concentration up to 1,400 mg L^{-1}), butyric (up to 850 mg L^{-1}) and caproic (up to 730 mg L^{-1}) acids, whereas the other acids were detected at much lower concentrations (lower than 200 mg L^{-1}).

Biogas production yield was much higher under mesophilic conditions (84±2 mL $_{H2}$ g_{VS}^{-1}) than under non-controlled temperature (41±4 mL $_{H2}$ g_{VS}^{-1}). These results were comparable

with those observed by fermentative batch tests on organic waste (Giordano et al., 2011) and by continuous mode on CW (Venetsaneas et al., 2009).

Maximum concentration of VFAs and biogas H_2 content (68 ± 4 %) was observed within the first 5 experimental days; therefore, the HRT of 5 d was applied for the acidogenic stage under mesophilic conditions of the two-stage anaerobic reactors (Table 2).

9.3.3. Continuous codigestion experiments

The feed was periodically prepared by combining S_{CW} and S_{CM} at a volumetric ratio of 50% and stored at 4°C. However, the feed showed slightly lower concentrations than the S_{CW} and S_{CM} average values probably due to the partial degradation of the easily biodegradable organic matter; the resulting feed characteristics are reported in Table 1.

pH occurring in the reaction media of the acidogenic and methanogenic stages of R2 and R3 were similar (Table 2), whereas R1 showed slightly acidic conditions. Therefore, the codigestion of CW and CM allowed the systems to maintain stable pH values at both stages.

Both two-stage processes seemed to show better sCOD removal compared with the one-stage reactor (Table 2); moreover, R3 seemed to reach slightly higher sCOD removal efficiency than R2 (although these differences were not statistically different). The average sCOD removal during the acidogenic stages of both two-stage reactors was found to be approximately 30%.

The total carbohydrate concentration in the effluents was consistently lower than 0.5 g L^{-1}, corresponding to removal yields that were always higher than 95% (Table 2).

Total VFAs accumulated in the acidogenic stage were 6.9 ± 0.15 g L^{-1} and 5.8 ± 1.68 g L^{-1} for R2 and R3, respectively; they were then decreased (to a total concentration of VFAs that was lower than 1.0 g L^{-1}) by acetotrophic methanogens in the methanogenic stage. On the contrary, total VFAs in R1 remained stable between 1.5 and 2.0 g L^{-1}. The main VFAs detected in the acidogenic stages were, acetic, caproic, butyric and propionic acids, while the effluents of the methanogenic stages were mostly composed of acetic acid.

The total concentration of VFAs measured in the present study was lower than those obtained by other studies treating CW and CM singularly, demonstrating that the codigestion of the two substrates greatly improved the degradation of VFAs. In fact, Ghaly (1996), using a two-stage reactor at HRT of 20 d, measured total VFA concentrations over 2.0 g L^{-1} and below 0.1 g L^{-1} for CW and CM, respectively, when used alone.

Table 1. Main characteristics of substrates and inocula (mean ± standard deviation).

	Inoculum			Substrate		
Parameter	**I_{OM}**	**I_{CM}**	**I_R**	**S_{CW}**	**S_{CM}***	**Feed****
Density (g mL^{-1})	1.0 ± 0.01	1.06 ± 0.02	1.02 ± 0.01	0.99 ± 0.13	0.99 ± 0.01	-
pH	-	-	-	5.0	7.9	7.1
sCOD (g L^{-1})	22.4 ± 1.5	12.0 ± 1.5	22.6 ± 3.0	58.5 ± 1.7	9.4 ± 0.1	35.2 ± 6.7
Tot carbohydrates (g L^{-1})	-	-	-	42.2 ± 2.8	1.3 ± 0.31	11.8 ± 4.2
Proteins (g L^{-1})	-	-	-	1.3 ± 0.4	0.4 ± 0.1	0.8 ± 0.2
TS (g L^{-1})	11.5 ± 0.2	23.2 ± 4.1	31.8 ± 3.8	57.8 ± 7.9	25.6 ± 0.1	36.3 ± 2.5
VS (g L^{-1})	4.9 ± 0.1	13.2 ± 2.8	14.6 ± 1.2	52.8 ± 7.6	17.6 ± 0.1	30.4 ± 3.6

* Sample diluted with water (1:2); ** S_{CW}:S_{CM} - 50:50 v/v

Methane production rate at steady state was generally stable in all three reactors. However, R2 and R3 showed methane production approximately 40% higher than R1 (Table 2). Moreover, although the biogas composition of the three reactors fell within the typical range for AD of agricultural waste (e.g. Comino et al., 2012), the two-stage systems also performed better than the one-stage process in terms of methane content (Table 2). It is of note that significant H_2 amounts were collected from the acidogenic stage of R2 (Table 2) due to the complete physical separation of the two stages, whereas the methane content was always below 5 %.

The methane yield and the methane concentration in the biogas were also higher in the two-stage reactors than in the one-stage reactor (Table 2). However, it is important to specify that the methane percentage of 63% detected in R2 was related to the methanogenic stage, whereas the CH_4 percentage (60%) of R3 was measured over both stages.

Table 2. Main results of the bench scale reactors.

	pH		Removal (%)		**Productivity (L L^{-1} d^{-1})			Composition (%)		Yield (L_{CH_4} kg_{VS}^{-1})
	Acidog.	**Methan.**	**sCOD**	**Carbohydrate**	**CH_4**	**H_2**	**Biogas**	**CH_4**	**H_2**	**CH_4**
R1	-	6.7 ± 0.5	71 ± 8	96	0.18 ± 0.04	-	0.38	48 ± 5	-	120
R2	4.9 ± 0.2	7.8 ± 0.4	80 ± 11	98	0.28 ± 0.05	0.1	0.42	*63 ± 7	*32 ± 4	233
R3	5.1 ± 0.3	7.5 ± 0.3	83 ± 6	98	0.31 ± 0.04	0.02	0.51	60 ± 6	2	258

*values measured in the biogas from the headspace of the two separate stages;
**values estimated on the volume of the entire systems

The results of the continuous experiments (Table 2) showed lower yields than those obtained in batch conditions (Fig. 2). However, the maximum methane yield of 320±9 $mL_{CH4}\ g_{VS}^{-1}$ achieved in batch conditions is related to "ultimate" biogas production (i.e. for complete substrate methanisation) that is obtained with a much longer test duration (50 d) than the HRT of the continuous experiments. Nevertheless, the yields obtained in this study are mostly in agreement with data recently reviewed by Esposito et al. (2012) and Nasir et al. (2012).
The results, therefore, demonstrate the much higher efficiency of the two-stage systems than the one-stage one treating CW and CM in codigestion.

9.4. Conclusions

The results demonstrate that the AD of CW and CM at 50% volumetric ratio provides higher biomethanisation yields than when the two wastes undergo the same process individually.
Moreover, the study demonstrates the much higher efficiency of the two-stage system rather than the one-stage system treating CW and CM in codigestion.
The concentric two-stage reactor obtained a slightly higher methane yield that could be explained by better use of the hydrogen produced in the acidogenic phase, which, with the lower footprint, could represent an improvement of AD for agroindustrial waste codigestion.

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Chapter 10

EFFECT OF CRUDE GLYCEROL CONCENTRATION ON 1,3-PROPANEDIOL PRODUCTION BY *Citrobacter freundii**

Abstract

The biodiesel industry is now dealing with an increasing amount of co-produced crude glycerol. Glycerol could be used as substrate for microbial conversion to 1,3-propanediol (PDO). The aim of this study was to evaluate the effect of different initial glycerol concentration on PDO production by *C. freundii* under mixing and static operating conditions (aerobic and micro-aerobic cultures, respectively). The study demonstrates the capacity of *C. freundii* to convert crude glycerol to PDO as the main product, up to the initial concentration of 150 g L^{-1}, achieving production yield of 0.46- 0.68 (mol of PDO on mol of fermented glycerol). Moreover, higher microbial activity and PDO production were obtained in aerobic than micro-aerobic cultures. Other end-products, mainly 2,3-butanediol and ethanol, were also detected although at much lower concentration than PDO.

Keywords: 1,3-propanediol, *Citrobacter freundii*, crude glycerol, fermentation, micro-aerobiosis.

* Paper in preparation for possible publication

10.1. Introduction

Biodiesel is considered one of the most promising sources of renewable fuels since it can decrease the dependency on petroleum and thus supports sustainable development (Almeida et al., 2012).

However, the great growth in biodiesel production of the last decade is causing a surplus of the coproduction of crude glycerol (cG) which has to be properly managed in order to improve the competitiveness of the biodiesel industry (Almeida et al., 2012; Maervoet et al., 2012; Yang et al., 2012).

Crude glycerol derives from the transesterification reaction of lipids (triglycerides) and presents great opportunities for biotechnological applications since it could be used, instead of the pure glycerol, as substrate for the biological synthesis of several high-value chemicals such as 1,3-propanediol (PDO), succinic acid (HSu), propionic acid, ethanol (EtOH), butanol, 2,3-butanediol (BD) and hydrogen (da Silva et al., 2009; Yang et al., 2012). Among these end-products, PDO is a very promising bulk chemical since it can be used for the synthesis of polyurethanes and polyesters; among these polymers, the polytrimethylene terephthalate (PTT) is of particular interest since it has important applications in many industrial sectors, such as food, pharmaceutical, or cosmetics (Clomburg and Gonzalez, 2013; da Silva et al., 2009; Maervoet et al., 2012;).

Although the synthesis of PDO by microbial fermentation is known since one hundred years, it has received attention over the last decade only, since PDO produced through biotechnological conversion of glycerol is now considered a sustainable chemical due to the use of a renewable feedstock (Clomburg and Gonzalez, 2013; Saxena et al., 2009).

Most of the studies on microbial PDO production have been carried out using pure glycerol and/or pure cultures (Saxena et al., 2009) and it is only over the last decade that several papers (Anand and Saxena et al., 2012; Chatzifragkou and Papanikolaou, 2012; Maervoet et al., 2012; Moon et al., 2010; Petitdemange et al., 1995; Yang et al., 2012;) have been published on direct utilisation of biodiesel-derived (crude) glycerol; however, these studies reported results that strongly depend on the substrate source, the used microorganism and the applied operating conditions.

PDO has been successfully produced through microbial conversion of crude glycerol under anaerobic conditions by genera belonging to Enterobacteriaceae and Clostridiaceae families,

such as *Klebsiella*, Enterobacter, *Citrobacter* and *Clostridium* (Almeida et al., 2012; Saxena et al., 2009).

Differently from the Clostridiaceae family that includes obligate anaerobes, the genera belonging to the Enterobacteriaceae family are facultative anaerobic and are able to produce PDO under anaerobic and aerobic conditions (Cheng et al., 2004; Hao et al., 2008; Maervoet et al., 2012; Metsoviti et al., 2012;). However, the effect of different operating conditions (i.e. aerobiosis/anaerobios) has not been entirely explored. Saxena et al. (2009) reported that PDO is produced from glycerol anaerobically, while under aerobic conditions the intermediate 3-hydroxypropionaldehyde (3-HPA), a microbial inhibitor, can be produced. On the contrary, Chen et al. (2003) reported an improvement of PDO production by *K. pneumoniae* when changing the fermentation from anaerobic to low aeration and/or micro-aerobic conditions.

Even though *Citrobacter* has not been largely investigated in the past, it is gaining interest over the last few years because of its high PDO productivity and since it can produce PDO under both aerobic and anaerobic conditions (Anand and Saxena, 2012; Hao et al., 2008;). Nevertheless, most of the studies on *Citrobacter* involved the use of pure glycerol under anaerobic conditions. For example, Maervoet et al. (2012) tested various *Citrobacter* species for their capacity to produce PDO from pure glycerol under anaerobic conditions. Although Metsoviti et al. (2012) recently tested several bacterial strains for their capacity to produce PDO under anaerobic and aerobic conditions, *C. freundii* was tested under anaerobic conditions only. However, Hao et al. (2008) demonstrated that *C. freundii* can produce PDO also under aerobic conditions and, therefore, strict anaerobic conditions are not needed.

Crude glycerol may contain numerous impurities (e.g. methanol, soaps, salts, esters, residual fatty acids, heavy metals) with variable composition due to the technological methods involved in the manufacturing biodiesel, which could greatly affect (or inhibit) the microorganisms metabolism (Almeida et al., 2012; Chatzifragkou and Papanikolaou, 2012; Papanikolau et al., 2008;). In fact, Anand and Saxena (2012) reported that *C freundii* can produce significant amount of PDO by crude glycerol only after substrate purification. Although various treating techniques have been reported for cG purification in order to obtain high-quality substrate for industrial applications (Anand and Saxena, 2012), they are still quite costly. Therefore, the valorisation of crude glycerol without any pre-treatment is still considered a major challenge (Almeida et al., 2012; Chatzifragkou and Papanikolaou, 2012; Clomburg and Gonzalez, 2013) to combine the management of the glycerol disposed of by

the biodiesel industry with the availability of low-cost renewable feedstock for industrial fermentation.

The aim of this study was to evaluate the effect of different initial cG concentration on PDO production by *C. freundii*. Moreover, the PDO production was also evaluated under two different operating conditions, as aerobic and micro-aerobic cultures.

10.2. Materials and methods

10.2.1. Microorganism, culture medium and glycerol

The microorganism investigated in the present study was *Citrobacter freundii* DSM 15979 obtained from DSMZ culture collection (Braun-schweig, Germany) as lyophilised culture.

The inoculum used throughout the fermentation experiments was stored in Tryptic Soy Broth (TSB) with 20% (w/v) glycerol (Sigma Chemical, St. Louis, MO, USA) at -20°C.

Before use, the microorganism was cultured in Tryptic Soil Agar (TSA) using the same TSB with the addition of agar (10 g L^{-1}) and then transferred to 100 ml flasks containing 30 mL±1 of TSB. Thereafter, the culture was maintained overnight under mixing conditions by using an orbital shaker (at 150±5 rpm) and temperature of 30±0.5°C, so that to reach the exponential growth. The resulting culture was used as inoculum of the experimental fermentation medium.

The experimental culture medium (CM) was composed by (per L of distilled water) 5.0 g K_2HPO_4, 2.5 g KH_2PO_4, 4.0 g NH_4Cl, 0.3 g MgSO4.7H_2O, 1.5 g yeast extract (Merck), 0.2 g $CaCl_2.2H_2O$, 0.01 g ml $FeSO_4.7H_2O$ and 1.0 mL of trace element solution. Trace element solution consisted (per L of distilled water) of 20 mg $CuCl_2$, 25 mg $MnCl_2.4H_2O$, 60 mg H_3BO_3, 35 mg $Na_2Mo_4.2H_2O$, 0.2 g $CoCl_2.2H_2O$ and 4.0 mL HCl (37%).

The experimental medium contained glycerol as sole carbon source. Crude glycerol supplied by a biodiesel production plant (located in Northern Italy) and the reagent-grade glycerol (Sigma-Aldrich, 99% w/w) used for comparison, were utilised as feed.

The sample of crude glycerol had a glycerol content of approximately 85 % (w w^{-1}), a density of 1.2 g L^{-1} and the methanol content was negligible.

10.2.2. Batch fermentations

The experiments were carried out under batch conditions. The batch fermentations were performed using 100-mL conical flasks containing 30±1 mL of working volume composed of CM added with glycerol as substrate. The flasks including CM supplemented with different concentrations of glycerol were plugged by cotton and then sterilised in autoclave (121^{o}C, 20 min) before use. The pH of CM was initially adjusted to value of 7.0±0.5 by NaOH solution (0.1 N) before autoclaving and remained un-controlled during the batch fermentation. The sterilised CM was inoculated with the exponential growing culture (see Section 2.1) at the volume ratio of 1:100 (v v^{-1}, culture: working volume).

Since the used strain is a facultative anaerobe, the batch experiments were conducted both under aerobic and micro-aerobic culture conditions. Aerobic culture was performed by placing fermentation flasks (plugged by cotton) on an orbital shaker (Adolf Kuhner AG, Basel, Switzerland) maintained at an agitation rate of 150±5 rpm; micro-aerobiosis was obtained by maintaining the experimental flasks under static condition.

The cultures were kept at 30±0.5°C using an incubator (Vismara 400 thermic line Laselettronic s.r.l., Italy).

The experiments were performed in duplicate and lasted at least 24 h. Some fermentations were monitored for prolonged time up to 72 h in order to assess any further metabolic activities.

10.2.3. Effect of crude glycerol concentration on PDO production

The optimal substrate concentration for PDO production by *C. freundii* was evaluated at increasing amount of crude glycerol.

The cG sample was tested in aerobic and micro-aerobic cultures at progressive concentrations of 5, 10, 20, 40, 60, 80, 100 and 150 g L^{-1}. These concentrations are theoretical values, while the measured concentrations are reported in the Results and Discussion Section (Section 3.2). Aerobic and micro-aerobic culture conditions were obtained as described in Section 2.2.

Batch tests were also carried out using pure glycerol (at two different concentrations of 20 and 80 g L^{-1}) in order to assess potential effects of the crude glycerol impurities on *C. freundii* growth (as optical density).

The experiment lasted until the complete depletion of the substrates. The microbial growth using pure and crude glycerol was compared in aerobic and micro-aerobic cultures.

10.2.4. Analytical methods

Samples from the fermentation flasks were collected at various time intervals for the measurements of the cell growth, glycerol (G), PDO, 2,3-butanediol (BD), ethanol (EtOH), succinic acid (HSu), lactic acid (HLa), acetic acid (HAc) and pH.

The cell growth was estimated as optical density (OD) at 600 nm using a UV-VIS spectrophotometer (Cary 100, Varian Inc., Italy).

Specific growth rate (μ) was estimated from OD values using the following equation (Vital et al., 2008),

$$\mu = \ln \frac{OD_t}{OD_{t-1}} * \Delta t^{-1} \qquad (1)$$

where OD_t and OD_{t-1} are the OD values at two time intervals and Δt is the time interval between the two measurements.

The pH of the cultures was measured using a pH meter (Thermo Orion Model 720A, Research Inc., Beverly, MA, USA) and a pH probe (Orion 81-04).

Glycerol, PDO, BD, EtOH, HSu, HLa and HAc were analysed by High-Performance Liquid Chromatography (HPLC) after centrifugation (J2-HS, Beckman Inc., USA) at approximately 7500 g for 15 minutes and filtration (0.22 μm, cellulose acetate filters). The HPLC system was equipped with a Phenomenex Rezex RHM Monosaccharide (H+) 300 x 7.8 mm ion exchange column and with a Thermo Surveyor Refractive Index Detector. The column and detector temperature were 65 and 40°C, respectively. A solution of H_2SO_4 (5 mM) was used as mobile phase at a flow rate of 0.6 mL min^{-1}, and the injection volume was 20 μL.

All results are presented as average of the data from the experiments ran in duplicate.

10.3. Results and discussion

10.3.1. *Growth of C. freundii on crude glycerol*

The potential inhibitory effect of the tested substrate on *C. freundii* was evaluated by comparing microbial growth on crude with pure glycerol (pG). At the concentration of 20 g L^{-1} no growth inhibition was observed (Fig. 1a) and complete depletion of glycerol

occurred within 6-8 h in aerobic cultures. Consequently, the microbial growth was evaluated at increased substrate concentration. At concentration of pure and crude glycerol of 80 g L^{-1}, only a slight difference of the OD trends was observed (up to 23 % after 24 h in micro-aerobic cultures) demonstrating that the tested crude glycerol is an effective substrate for *C. freundii* growth (Fig. 1b).

Other authors (Gonzalez-Pajuelo et al., 2004) confirmed no significant inhibition on the anaerobic growth of *Clostriudium butyricum* fed with cG at similar concentration used in the present study.

C. freundii showed much higher specific growth rates under mixing condition than static one both for pure and crude glycerol. The maximum specific growth rate (μ_{MAX}) estimated at the concentration of 80 g L^{-1} were 0.62 h^{-1} for pG and 0.91 for cG in aerobic cultures meanwhile in micro-aerobiosis the measured μ_{MAX} decreased to 0.60 and 0.45 h^{-1} for pG and cG, respectively, suggesting that mixing condition improved the cell growth and in particularly on crude glycerol. It is of note that very low differences of the final OD values (Fig. 1) and of the estimated μ_{MAX} were observed between pure and crude glycerol in aerobic cultures.

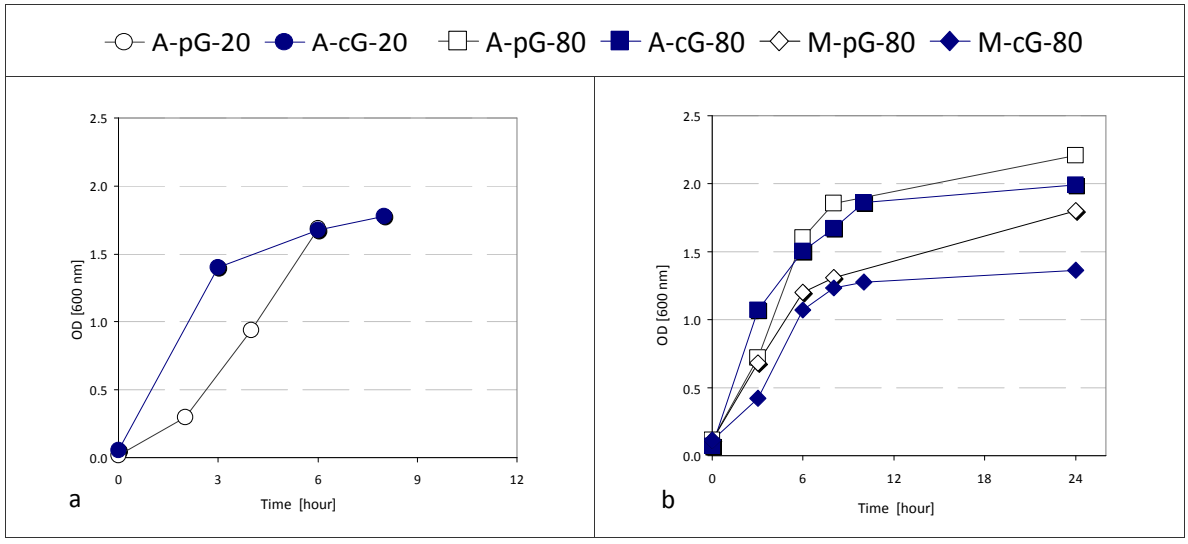

Figure 1. Growth of *C. freundii* on pG and cG at initial glycerol concentration of 20 (a) and 80 g L^{-1} (b); A, aerobic cultures; M, micro-aerobic cultures.

Maervoet et al. (2012) reached the maximum growth rates up to 0.40 h^{-1} using *Citrobacter werkmanii* on 20 g L^{-1} of pure glycerol under anaerobic conditions. However, in this study

higher growth rates were observed probably due to the different operating conditions (mixing) and the use of a different microorganism.

Since no significant differences on the growth rate between the use of pG and cG was observed, the microbial growth was investigated at different crude glycerol concentration in aerobic and in micro-aerobic cultures.

Contrary to Anand and Saxena (2012) who reported growth inhibition of *C. freundii* by using crude glycerol (at 50 g L^{-1}), in this study the impurities of the crude glycerol did not significantly affect the *C. freundii* metabolism, as demonstrated by the OD curves for all tested glycerol concentrations (Fig. 2a and 2b).

In fact, the results demonstrated that the growth capacity of *C. freundii* was only slightly affected by the tested concentrations of crude glycerol (Fig. 2); only the highest concentration tested (150 g L^{-1}) showed evident inhibition of the microbial growth. The results of the batch experiments at different cG concentrations confirmed that the estimated maximum growth rate (Fig. 2c and 2d) was much higher in aerobic (0.6-1.1 h^{-1}) than micro-aerobic cultures (0.35-0.45 h^{-1}).

It is of note that the microbial growth rate was, however, significantly affected by the increased cG concentration and by the operating (mixing and static) conditions. In fact, while the growth rate measured during the first three h (μ_{3h}) batch experiments decreased with the increased cG concentration (Fig. 2c and 2d), the opposite trend was observed for the same kinetics measured within 3-6 h (μ_{6h}).

These trends were even more apparent under static conditions where the growth rate measured within 3 h and within 3-6 h presented similar values (Fig. 2c and 2d). Therefore, since almost the same OD was achieved after 24 h (Fig. 2a and 2b) while differences were observed in the growth rate (Fig. 2c and 2d), the increase of initial crude glycerol concentration seems to mainly affect the growth kinetic rather than the growth yield. Moreover, Figure 2c and 2d seem to indicate that the increase of the cG concentration could prolong the growth lag-phase. In addition, it is of note that longer lag-phases seemed to be favoured by static condition (Fig. 2d) since very lower growth rates were detected after six h (μ_{3h}) of the experiment in micro-aerobic cultures compared to aerobic ones.

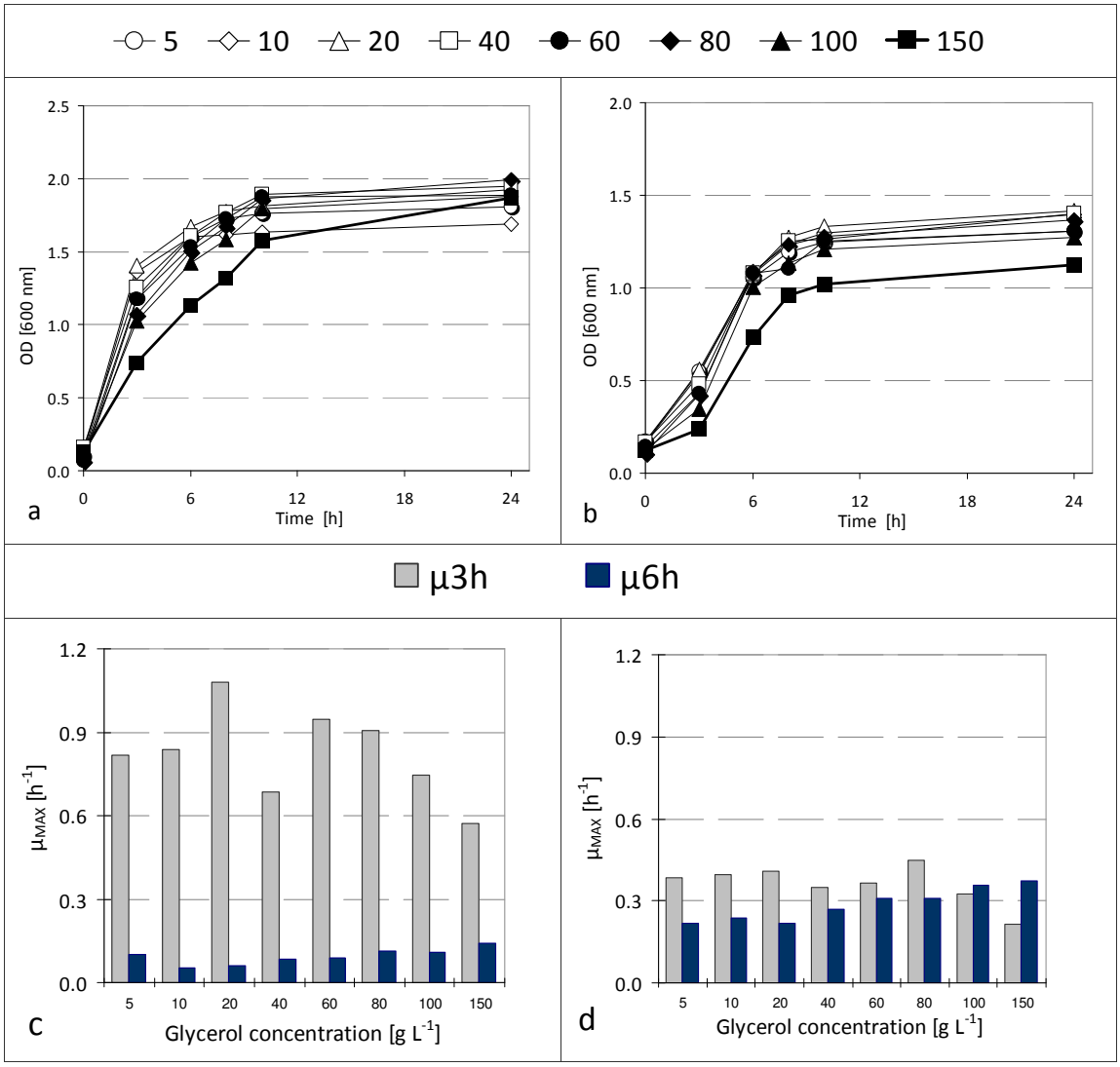

Figure 2. Effect of initial cG concentrations on *C. freundii* growth: OD values of aerobic (a) and micro-aerobic (b) cultures; growth rate (μ) within 0-3 h (μ3h) and 3-6 h (μ6h) in aerobic (c) and micro-aerobic cultures (d).

The optimal growth rates obtained in this study are slightly higher than the data reported by Kaur et al. (2012) which cultivated *Clostridium diolis* under anaerobic conditions and by Sattayasamitsathit et al. (2011) which worked with *Klebsiella pneumoniae* under micro-aerobic conditions, confirming that mixing conditions improved microbial growth. On the contrary, the growth rates obtained in micro-aerobic cultures are comparable with previous studies under similar operating conditions (Sattayasamitsathit et al., 2011).

A longer lag-phase (10–11 h vs. 5 h at lower concentrations) was also observed by Kaur et al. (2012) in anaerobic cultures when grown on high concentrations (100 g L^{-1}) of crude glycerol, confirming that the cG concentration can significantly affect the microbial kinetics.

10.3.2. PDO production

The results of the batch experiments demonstrated that *C. freundii* produces PDO using cG as substrate both in aerobic and micro-aerobic cultures (Fig. 3). However, residual glycerol was observed at the concentrations of 60 and 40 g L^{-1} after 24 h of batch fermentation in aerobic and micro-aerobic cultures, respectively (Fig. 3a and 3b). It is of note that the higher the initial substrate concentration, the higher the residual glycerol, demonstration that there was a maximum utilisation yield.

Fig. 3a and 3b show that the initial glycerol concentration was approximately similar to the expected theoretical value for most of the batch tests.

Some experiments were prolonged up to 72 h in order to assess if the glycerol utilisation can continue; these experiments (data not shown) demonstrated that only a small fraction of the residual glycerol was further utilised by the microorganism.

Much higher concentration of residual glycerol was observed with initial concentration of 60-80 g L^{-1} in micro-aerobiosis than in aerobic cultures, demonstrating, as for microbial growth (see Section 3.1), that glycerol metabolism seems to be improved by mixing conditions (Fig. 3a and 3b).

Residual glycerol accumulation by *C. freundii* in anaerobic cultures was also observed by Barbirato et al. (1998) and Anand and Saxena (2012) at substrate concentration of 50 and 70 g L^{-1}, respectively: however, these studies did not investigate different initial substrate concentrations.

C. freundii was able to produce PDO at all the considered cG concentrations (Fig. 3c and 3d). However, the PDO synthesis was greatly affected by the initial substrate concentration.

The PDO production increased with the increasing cG concentration up to 80 and 60 g L^{-1} under the aerobic and micro-aerobic operating conditions, respectively, and then decreased with further increase of the substrate (Fig. 3c and 3d). The highest concentration of PDO measured after 24 h of batch fermentation was 43 g L^{-1} and 25 g L^{-1} for the aerobic and micro-aerobic cultures, respectively.

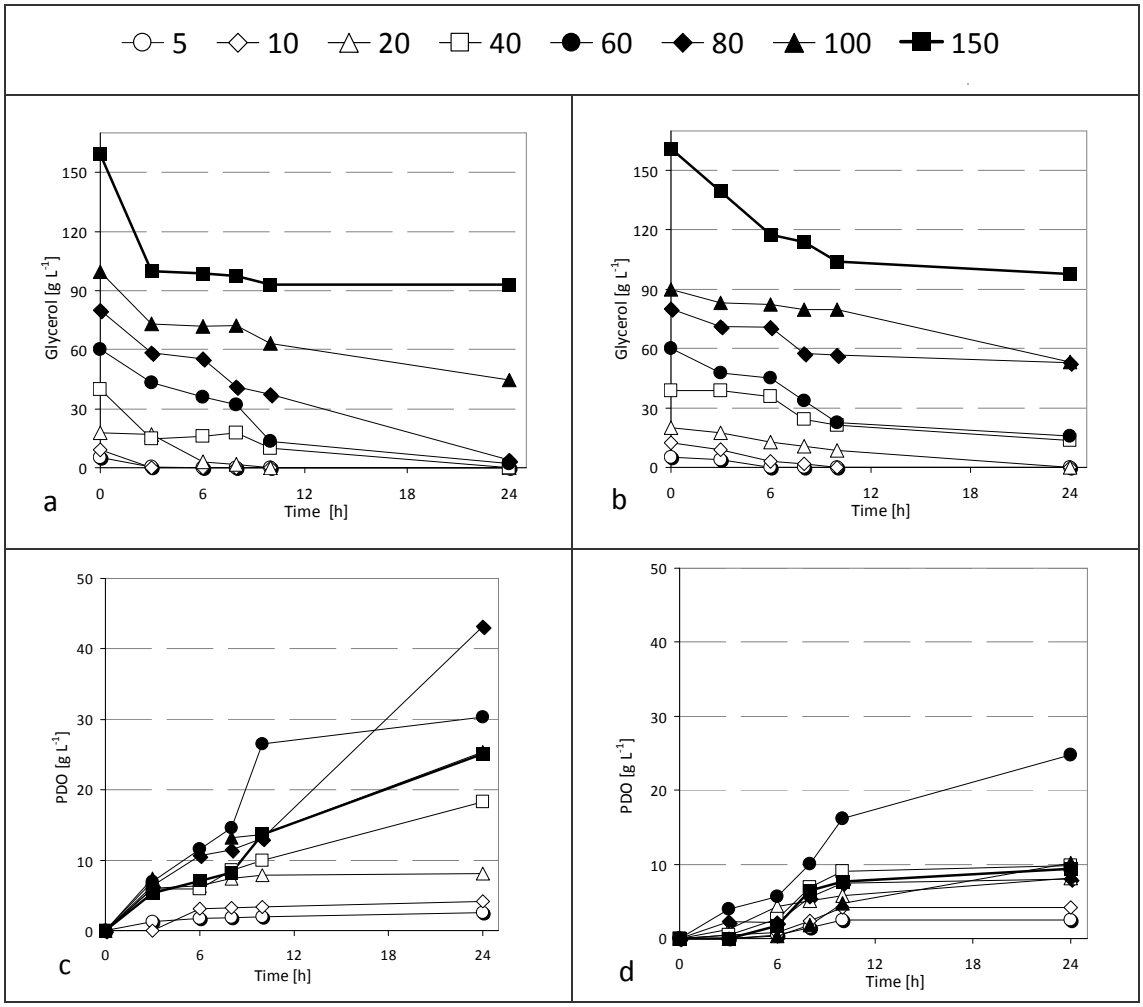

Figure 3. Effect of initial crude glycerol concentrations on PDO production by C. freundii: concentrations of residual glycerol in aerobic (a) and micro-aerobic (b) cultures; cumulative PDO production in aerobic (c) and micro-aerobic (d) cultures.

Moreover, slight higher PDO concentrations were obtained in aerobic than in micro-aerobic cultures, confirming once more the improved metabolism by mixing condition.

The results demonstrated that there was an optimal cG concentration for microbial production of PDO by *C. freundii*. Other studies also reported an optimal initial concentration for PDO production. For example, Sattayasamitsathit et al. (2011) individuated the optimal initial concentration of crude glycerol in the range between 60 and 80 g L^{-1} for conversion to PDO by *Klebsiella pneumoniae*. Similarly, other researchers (Barbirato et al., 1998; Zheng et al., 2008) reported an optimal initial concentration of glycerol for PDO production also using other microorganisms as *Enterobacter agglomerans* and *Klebsiella pneumoniae*.

Therefore, high cG concentration showed to inhibit PDO production, Sattayasamitsathit et al. (2011) explained the inhibitory effect as due to the presence of impurities in the substrate and to the production of other intermediates (e.g. polyhydroxyalkanoates) which accumulated when high concentration of crude glycerol was used. The experiments prolonged to 72 h did not improve the PDO production (data not shown), confirming that potential inhibition occurs at high cG concentration.

The different PDO production and residual glycerol greatly affected the yield of the process estimated as molar ratio of mol of product over the fermented glycerol (Fig 4).

Figure 4 compares the residual glycerol and the produced PDO with their relative yields of fermentation and PDO production at the applied initial cG concentrations both in aerobic and micro-aerobic cultures.

Figure 4 clearly shows that higher residual glycerol concentrations were obtained in micro-aerobic cultures resulting to low PDO productions (Fig. 4a and 4b). However, although different fermentative yields (Y_F) were obtained in aerobic and micro-aerobic cultures, the PDO production yields (Y_P) were more comparable under the two applied operating conditions (Fig. 4c and 4d), demonstrating that the mixing seems to affect the microbial kinetics more than the metabolic yields as also observed for the microbial growth as reported in section 3.1.

It is of note that Y_F values measured at low cG concentration (when complete substrate depletion occurred in both conditions) and at the maximum one (i.e. 150 g L-1) were the same in aerobic and in micro-aerobic cultures. Therefore, the cG utilisation at high concentration seemed to be more affected by the substrate (i.e. substrate inhibition) than by the mixing or static operating conditions.

The Y_P values measured in aerobic cultures were rather stable irrespective of the applied substrate concentration and applied conditions, ranging from 0.46-0.68 mol_{PDO} $mol^{-1}_{ferm.cG}$. Although the Y_P was more variable in micro-aerobic (0.18-0.68 mol_{PDO} $mol^{-1}_{ferm.cG}$) than in aerobic cultures, the maximum yield obtained were the same under the two operating conditions (Fig. 4c and 4d), confirming that the achievable PDO yield seems to be not greatly affected by the operating conditions (i.e. aerobiosis). The maximum Y_P was obtained at intermediate cG concentration to demonstrate that there is an optimal substrate concentration for PDO production (Sattayasamitsathit et al., 2011).

It has to be highlighted that the lowest yields were obtained at the highest substrate concentration, confirming, once again, that at cG could be inhibitory to the microbial PDO production process.

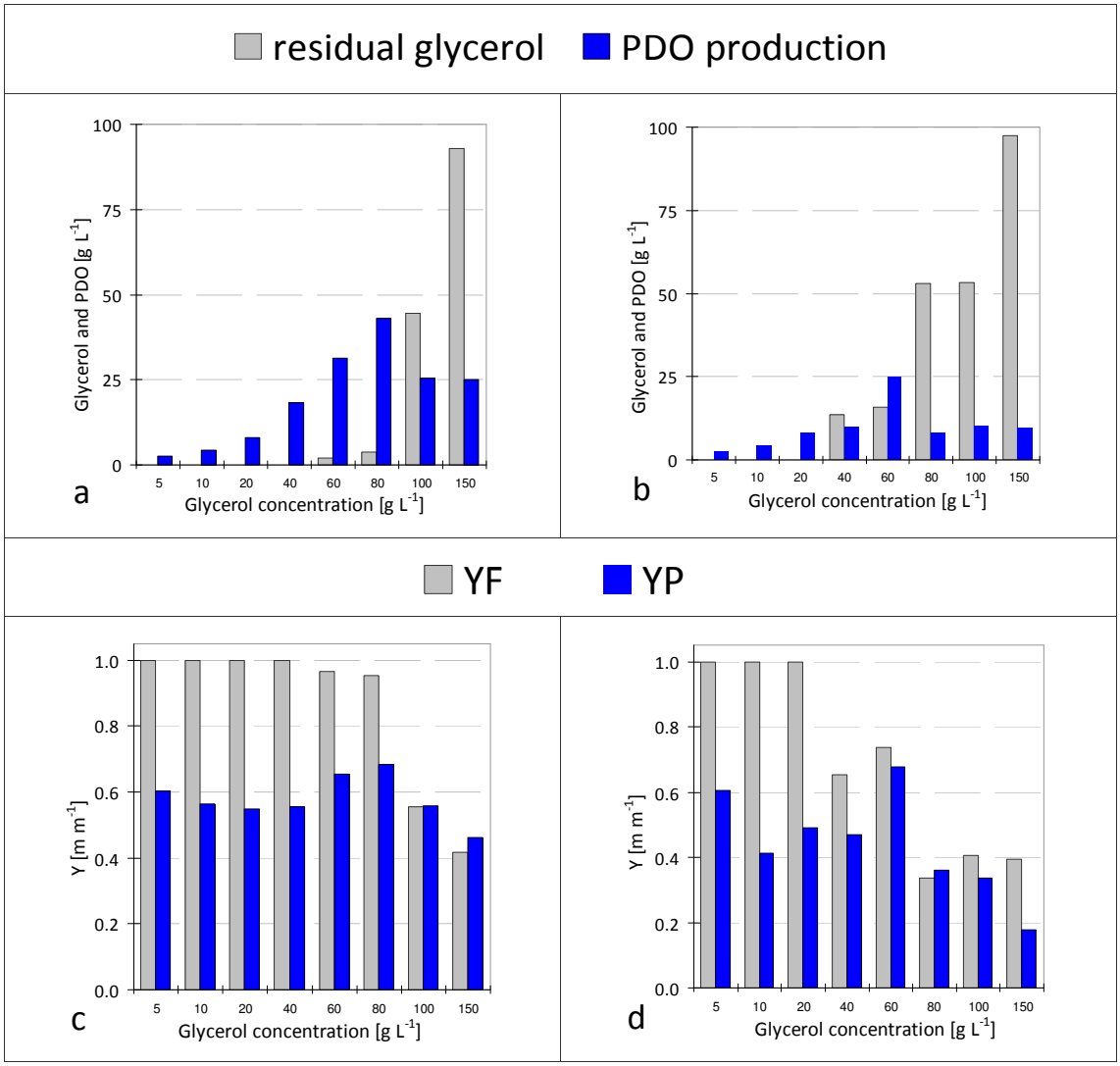

Figure 4. Concentrations of residual glycerol and produced PDO in aerobic (a) and micro-aerobic (b) cultures; yield of fermentations (Y_F) and of PDO production (Y_P) in aerobic (c) and micro-aerobic (d) cultures.

Therefore, although the direct use of crude glycerol is desirable, this study confirms that very high concentration of glycerol from the biodiesel industry could inhibit the microbial processes for PDO production (Anand and Saxena, 2012; Barbirato et al., 1998; González-Pajuelo et al., 2004; Moon et al., 2010; Sattayasamitsathit et al., 2011; Zheng et al., 2008).

However, our results showed that PDO production can be sustained by *C. freundii* through the optimisation of operating conditions.

The obtained values of Y_P were well inside the range (0.50-0.69 mol mol^{-1}) for PDO production by different microorganisms and were slightly higher than those reported in literature for anaerobic fermentation of pure glycerol by *C .freundii* (Anand and Saxena, 2012; Metsoviti et al., 2012; Saxena et al., 2009). Our results are therefore close to those obtained by similar batch fermentation experiments of crude glycerol using *K. pneumoniae* (Mu et al., 2006; Sattayasamitsathit et al., 2011), which is considered one of the most effective microorganism for PDO production (Hao et al., 2008; Liu et al., 2010).

10.3.3. Other fermentative products of glycerol

During the batch tests, other end-products of the cG fermentation were detected.

The maximum concentrations (not the concentration at the end of the batch experiments) of the end-products as, Hsu, HLa, HAc, BD and EtOH, measured in aerobic and micro-aerobic cultures are shown in the Figure 5. The results confirm that PDO was the main end-product of the fermentative process since its concentrations (Fig. 4) were well above those of the other chemicals (Fig. 5). Among the other end-products BD presented the highest concentrations reaching the maximum values of 17 g L^{-1} under aerobiosis. It is of note that, similarly to PDO production, its concentrations showed the highest values at initial cG of 60-80 g L^{-1}, and higher content (approximately of 50%) were measured in aerobic than in micro-aerobic cultures (Fig. 5a and 5b).

EtOH was also detected at significant concentrations (up to 6 g L^{-1}).

On the contrary, the organic acids (HLa, HAc, Hsu) were produced at much lower concentrations than the other end-products, with the exception of HAc which presented some sporadic significant peaks (Fig. 5).

The maximum yields of BD production were approximately of 0.25 to 0.30 (mol_{BD} $mol^{-1}_{ferm.cG}$) that are in agreement with the results obtained by Metsoviti et al. (2012) using *C. freundii*.

Nevertheless, it has to be highlighted that the trends of these end-products were very different than the behaviour of PDO. In fact, while the produced PDO remained in the culture medium, BD and EtOH were degraded within the 24 h of the batch test with the exception for BD at the highest cG loads (Fig. 5c and 5d). This behaviour could be due to the increased residual

glycerol content which hinders the consumption of BD or to an inhibitory effect of cG at the highest concentrations.

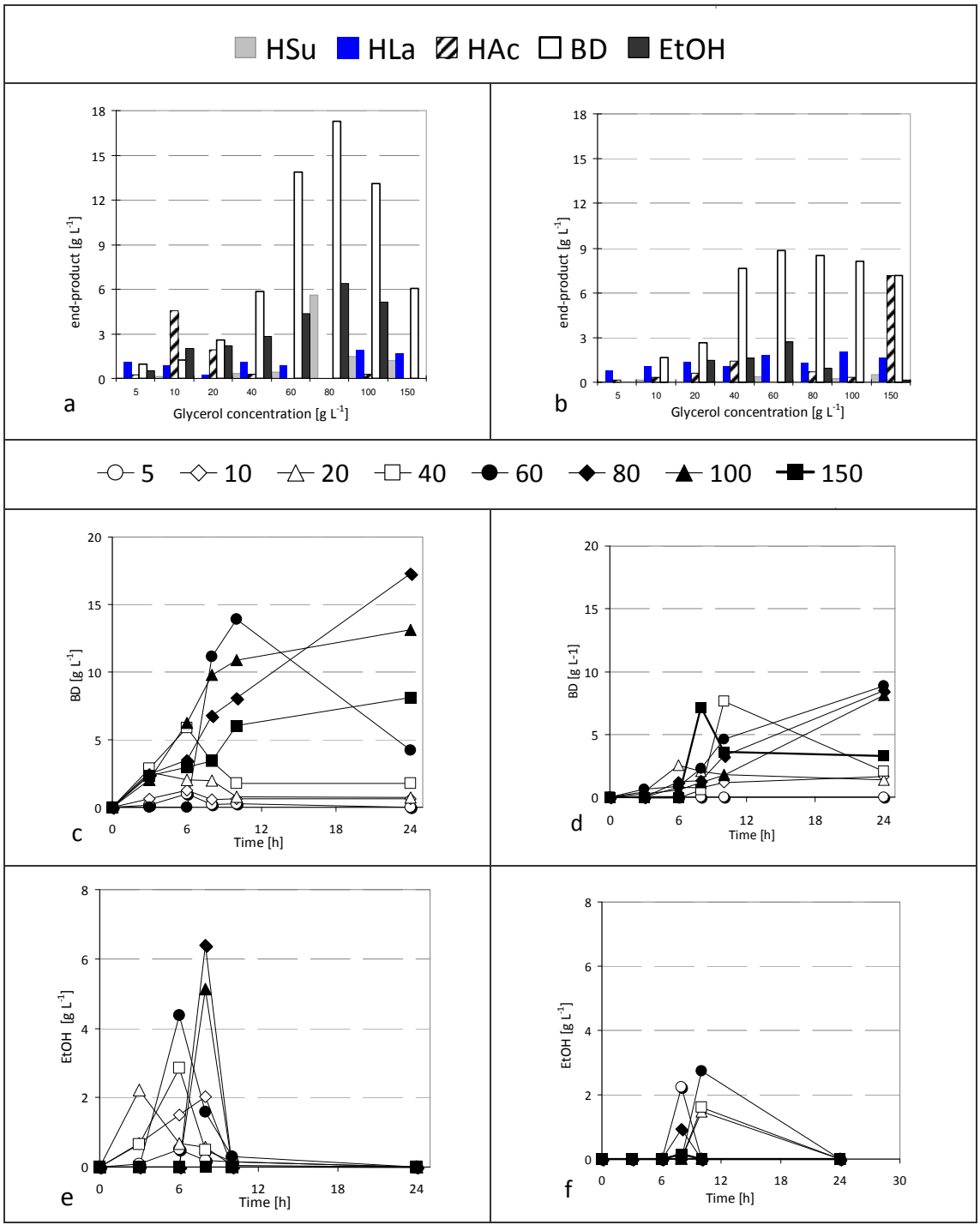

Figure 5. Other measured end-products at different initial glycerol concentrations: maximum concentrations in aerobic (a) and micro-aerobic (b) cultures; BD trends in aerobic (c) and micro-aerobic cultures (d); EtOH in aerobic (e) and micro-aerobic cultures (f).

The depletion of these end-products in the batch experiments could lead to the productions of other metabolites (Almeida et al., 2012) involved in the glycerol fermentation, which were however not monitored.

10.4. Conclusions

Although several authors have reported that crude glycerol could severely inhibit the microbial metabolism and PDO production by *C. freundii* and by other microorganisms (Anand and Saxena, 2012; Moon et al., 2010), the present study demonstrated the capacity of *C. freundii* (DSM 15979) to grow on cG and to efficiently convert the substrate to PDO as the main product, up to the initial glycerol concentration of 100 g L^{-1}. However, at initial cG concentration higher than 100 g L^{-1}, a significant inhibition on *C freundii* growth was observed.

Other end-products (mainly BD and EtOH) were also detected during glycerol fermentation although at much lower concentration than PDO.

The obtained Y_P of 0.46-68 (mol mol^{-1}) of PDO on fermented glycerol were well in agreement with other studies using other well-known PDO-producing microorganisms (Metsoviti et al., 2012; Sattayasamitsathit et al., 2011).

The study also showed that higher microbial activity and PDO production were obtained in aerobic than in micro-aerobic cultures indicating that the mixing regime greatly improved the fermentative process.

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Chapter 11

GENERAL CONCLUSIONS

Innovative biotechnologies as environmental remediation strategies have been successfully implemented.

The investigated biotechnological solutions reached optimal performances of treatment and conversion of different organic waste and wastewater resulting suitable to replace or to improve the current technologies.

The biological applications were mainly addressed to anaerobic digestion by using fermentative processes which operated in different ways based on the available substrate, specific microbial process and target products.

The fermentative biotechnologies, by implementing innovative designs and enhanced processes, reached high yields of energy and bio-chemicals production with the simultaneous degradation or valorisation of the substrate.

Since the expansion of White Biotechnologies is rapidly increasing and the economic competitiveness of biofuels is mainly influenced by the cost of the available fuels, the studied fermentative processes, being environmentally friendly, could represent an improvement of the biorefinery system in order to increase the competitiveness and, thus, promote the use of renewable bioenergy.

Therefore, the proposed solutions proved to be suitable as White Biotechnologies for the remediation of environmental issues considered in this thesis.

The conclusions of the four research lines (see Chapter 1) can be summarised as follow.

The feasibility of combined biological processes, under anaerobic-anoxic and aerobic conditions, with filtrations techniques for the treatment of wastewater from textile industry was demonstrated.

Anaerobic MBR applied as pre-treatment reached very high decolourisation performance of wastewater containing azo dyes, whereas filtration post-treatments (i.e. NF) obtained high water quality standard suitable for reuse in the textile factory. The results also demonstrated that by combining diverse treatment processes, the effluents from different manufacturing

processes can be handled separately.With this aim, water quality criteria of wastewater based on the level of pollution were developed for their treatment and re-use in the companies.

In addition, the anaerobic process also allowed us to obtain significant methane productions.

In today's context of the anaerobic waste management, the case studies of landfill operated as bioreactor with recirculation of the produced leachate (as described in the research line 2), demonstrated high efficiency in the stabilisation of the landfilled material.

The proposed biotechnologies resulted effective for waste management. The anaerobic processes were able to combine biogas production with the degradation of organic matter. Moreover, the addition of oxidised nitrogen seemed to support ammonia removal, which could be obtained via the Anammox process.

The valorisation of agro-zootechnical industrial waste (i.e. dairy waste) through fermentative processes was demonstrated by an economic biotechnology for the treatment of organic waste and the production of renewable energy.

This case study demonstrated that the codigestion of cheese whey and dairy manure can be optimised by combining the conventional fermentative processes with the high efficiency of an innovative biotechnology designed for enhancing performance and reducing footprint.

It is noteworthy, that fermentative processes reached high biomethanation yields.

The results of the research line 4 demonstrated that crude glycerol, an important waste of the biodiesel industry, can be effectively converted to the bio-chemicals 1,3-propanediol by a microbial pure culture of *C. freundii*. The optimal conversion yields of crude glycerol to 1,3-propanediol obtained in this study are encouraging for the production of the target bio-chemical.

List of the abbreviations

AD: Anaerobic Digestion

AOP: Advanced Oxidation Process

BD: 2,3-butanediol

BMP: Biochemical Methane Potential

cG: crude glycerol

CM: cattle manure (in Chapter 9)

CM: culture medium (in Chapter 10)

COAG. Coagulation process

COAG. + UF (hollow fiber);

COD: Chemical Oxygen Demand

CW: Cheese whey

EtOH: Ethanol

GC: Gas Chromatograph

HAc: Acetic Acid

HBu : n-Butyric Acid

HLa: Lactic Acetic

HRT: Hydraulic Retention Time

HSu succinic acid

LBR: Landfill BioReactor

MBR: Membrane Bioreactors

MSWFF: Municipal Solid Waste Fine Fraction

NF: Nanofiltration

DO: Dissolved Oxygen

OD: Optical Density (in Chapter 10)

OD4-7: Oxygen Demand after four and seven days of respiration test (in Chapter 7)

OLR: Organic Loading Rate

OM: Organic Matter

PDO: 1,3-Propanediol

pG: pure Glycerol

SMEs: Small and Medium Enterprises

SRF: Solid Recovered Fuel

TKN: Total Kjeldahl Nitrogen

TOC: Total Organic Carbon (TOC)

TS: Total Solids

TSS : Total Suspended Solids

TXTWW: Textile WasteWater

UF: UltraFiltration

VFA: Volatile Fatty Acid

VS: Volatile Solids

VSS: Volatile Suspended Solids

Y_F: Fermentation Yeld

Y_P: 1,3-Propanediol production Yeld

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