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Assessing the potential phytotoxicity of digestate from winery wastes

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ABSTRACT

In this study, digestate from winery wastes was investigated focusing on phytotoxicity using macrophytes and evaluating the potential contribution of ammonium and copper. Spreading of digestate on soil could represent a suitable approach to recycle nutrients and organic matter, creating an on site circular economy. In this study, digestate quality was evaluated considering both chemical-physical characteristics and biological toxicity applying germination test. The effluent did not meet the entire amendment quality standard defined by Italian law (Decree 75/2010 germination index > 60% with solution of 30% v/v of digestate), but bio-stimulation was observed at low doses (3.15–6.25% v/v) for *S. alba* and *S. saccharatum*. The beneficial concentration agreed with Nitrate Directive dose and suggested that limited addition of digestate could have several positive effects on soil characteristics and on crop growth. Specific test using ammonium and copper solutions showed that these pollutants were not directly correlated to observed phytotoxicity.

1. Introduction

Anaerobic digestion (AD) has been widely diffused in the last decades to treat several type of organic waste such as organic fraction of municipal waste (Jain et al., 2015), waste activated sludge (Appels et al., 2008), livestock effluents (Ward et al., 2008) and winery wastes (Da Ros et al., 2016a). The effluent of AD process is called digestate and its recovery can increase the economical and environmental process sustainability. The direct application of digestate to soil is currently considered an inexpensive option for its disposal and for recovery of their mineral and organic constituents for agricultural systems (Alburquerque et al., 2012). In fact, during the anaerobic process, part of organic nitrogen is transformed into ammonium, while phosphorus is partially converted in orthophosphate; both these chemicals are easily available for plants growth. Digestate application can consequently substitute or reduce the use of chemical fertilizer, though the amount must be calculated according with the Nitrate Directive (Directive 91/676/EEC). Considering the organic constituents, the labile fraction was mostly degraded during the AD process and lignin-like material, complex lipids and steroids became concentrated (Lorenz et al., 2007) reported that these compounds are humos precursors, consequently supply organic carbon in the soil. Moreover application of digestate leads to enhanced microbial processes such as nitrogen mineralization and ammonia oxidation (Abubaker et al., 2012; Odlare et al., 2008), and enzymatic activity (Galvez et al., 2012), which further increases the long-term nutrient release in soils (Abubaker et al., 2012; Odlare et al., 2008). Digestate improves soil physical properties (Różyło et al., 2015) increasing water balance and soil structure (Abubaker et al., 2012). In spite of digestate beneficial properties, it has to meet also quality standards in terms of heavy metals, polychlorinated byphenyls (PCBs), pathogens and phytotoxicity. Phytotoxicity is an interesting parameter evaluating the real digestate spreading impact on crops and it represents an index of its overall ecotoxicological impact. In fact the combined effect of the different contaminants mixed together, as well as their bioavailability, is difficult to estimate by chemical analysis while biological assays could supply the missing information (Alvarenga et al., 2007). Additionally, efforts should be made to identify the doses that will produce the desired fertilization effects ensuring the safety of agro-ecosystems (Różyło et al., 2015).

To date, many countries introduced germination index (GI) to assess the quality of amendment as the result of the combination of macrophytes germination and root elongation. Generally it is an indicative

Abbreviation: AD, anaerobic digestion; AS, Activated Sludge; COD, chemical oxygen demand; CSTR, continuous stirred tank reactor; D1 and D2, digestate samples 1 and 2; GAE, gallic acid equivalent; GI, germination index; HRT, hydraulic retention time; OLR, organic loading rate; TS, total solid; VS, volatile solids; EC, electrical conductivity; pCOD, particulate COD; sCOD, soluble COD; SRT, sludge retention time; TKN, total Kiendhal nitrogen; P_{tot}, total phosphorus

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limit value is provided in existing guidelines but only in Italy is a parameter enforced by law. The threshold for digestate acceptability as amendment according to the Italian legislation (D.Lgs 75/2010) was set at GI ≥ 60% in a digestate samples diluted at 30%.

GI was chosen for its simplicity, short time requirement (up to 72 h) and sensitivity, being the germination phase strongly affected by environmental conditions (Wang, 1991). It was applied mainly to compost (Komilis and Tziouvaras, 2009; Teglia et al., 2011a; Young et al., 2016) and recently to digestate (Di Maria et al., 2014; Pivato et al., 2016). Phytotoxicity test uses a matrix-based approach that considers the overall source of pollutants in the matrix and toxicants interaction. In most studies, it is applied as an indirect test, using an extract of the solid sample to identify its impact (Alvarenga et al., 2007) and the results depend strongly on the solid-to-liquid ratio assumed. Instead direct test deals with the raw sample (Kapanen and Itävaara, 2001) and gives more realistic results, because all kind of interactions between contaminants, soil matrix and test organisms are included and all site specific effects are integrated.

The presence of so many complex chemicals in the digestate (e.g. including metal ions, macro and micro-nutrients, organic pollutants) caused ecotoxicological interactions varying from synergism to antagonism (Gupta and Kelly, 1990), making toxicity etiology difficult to identify (Tam and Tiquia, 1994). Generally, phytotoxicity test carried out on digestate from livestock effluents showed stimulation at high dilution rate (Alburquerque et al., 2012; Pivato et al., 2016), while high concentrations showed germination inhibition. In contrast Gell et al. Gell et al. (2011) did not observe any differences from the control using digestate deriving from cow manure, pig slurry and human excreta, and three plant species (*Lactuca sativa L.*, *Raphanus sativus L.* and *Triticum aestivum, L.*). Germination index is usually inversely correlated with conductivity and ammonium concentration (Alburquerque et al., 2012; Tam and Tiquia, 1994; McLachlan et al., 2004). High ammonium concentration can reflect potential phytotoxicity (Teglia et al., 2011b; Tigini et al., 2016; Wong et al., 1983), but a threshold limit is not well defined. Di Maria et al. (2014) reported that concentration of 16–25 g $N\text{-}NH_4^+$/kgTS inhibited seed germination in *Lepidium sativum*, while Tigini et al. Tigini et al. (2016) indicated that the inhibiting concentration was higher than 2000 mg/L of $N\text{-}NH_4^+$ for *Lepidium sativum* and *Cucumis sativum*.

Salinity limits the germination of many plant species through osmotic effects or through ion toxicity (Brenchley and Probert, 1998). It is reported by Boluda et al. (2011) that salinity levels higher than 2.0–2.6 mS/cm can inhibit the number of *Lactuca sativa* germinated seeds and delay the germination process. Germination inhibition correlated by high conductivity level in the digestate was detected by several authors (Alburquerque et al., 2012; Pivato et al., 2016; Tigini et al., 2016). It can be associated with high concentration of sodium, chlorine, ammonium, and also metals. About metals in digestate, copper (Cu) and zinc (Zn) are the most recurrent (Alburquerque et al., 2012; Teglia et al., 2011a).

Phytotoxicity is not only correlated to chemical characteristics, but it depends on i) type of feedstock, ii) AD operational conditions (Abubaker et al., 2012; Tambone et al., 2010) and iii) macrophyte species used during the experimental phase. Di Maria et al. (2014) demonstrated that operational conditions could affect toxicity, in particular high organic loading rate (OLR) and short hydraulic retention time determined higher concentration of volatile fatty acids (VFAs), reducing the biological stability and, hence, the digestate germination index.

Considering the several parameters affecting digestate phytotoxicity, prediction of residual toxicity is difficult and experimental tests have to be carried out taking in consideration chemical characteristics and operational AD conditions.

Winery wastes are interesting substrates for AD in wine producing countries because of their high biodegradability and pilot-scale experimentation showed that *meso*philic process is the easiest to manage using hydraulic retention time higher than 20 days and organic loading rate of about 3 kg COD/m^3d (chemical oxygen demand, COD) (Da Ros et al., 2014a). Digestate spreading on vineyards could represent a suitable approach to recycle nutrients and organic matter creating an on site circular economy, but the phytotoxicity evaluation has never been made.

In this study, digestate from winery wastes was investigated focusing on phytotoxicity with macrophytes looking for the potential contribution of ammonium and copper.

2. Material and methods

2.1. Digestate production and sampling

Two winery wastes, called D1 and D2, were considered: D1 was waste activated sludge (AS) from winery wastewater treatment and D2 was wine lees. They were collected in a cellar in Conegliano (Italy) producing about 30,000,000 L of wine per year. The 75% of sold wine is white one and most of it is producing by Charmat method along the whole year. Throughout the year it generates 1.6 kg of wine lees and 2.0 L of wastewater per L of wine. The wastewater has high COD concentration (3747 mg/L in average) and was treated inside the cellar borders by conventional activated sludge (AS) process. As reported by Da Ros et al. (2016a), the AS process operated with average hydraulic and sludge retention times (HRT and SRT) of 6.7 d and 35 d, respectively. The oversized biological reactor volume allowed to operate with long HRT and SRT values, in order to withstand the load picks. The MLVSS was 3010 mg/L and the corresponding food to microorganisms' ratio was 0.26 kg COD/kg MLVSS per day. The COD was completely removed (95%) during the treatment and, in turn, 613 kg of dewatered waste AS was produced weekly. The substrate characteristics were reported in supplementary material and described in detail by Da Ros et al. (2016b).

A continuous stirred tank reactor (CSTR) with a working volume of 0.23 m^3 was employed for anaerobic co-digestion of waste AS and wine lees. The temperature was maintained at 37 °C using an external jacket. PT100 probes (OMEGA Engineering Inc., Norwalk, CT, USA) monitored the temperature trend during process and managed the water recirculation pumps. The reactor operated with an organic loading rate of 3.2 kg/(m^3 d) of chemical oxygen demand (COD) and HRT of 23 d. The organic load distribution between the two co-substrates considered the real waste flow characteristics: 80% of wine lees and 20% of waste AS.

The operational conditions were reached by a long start-up period (140 d) that consisted in slowing the increase of organic loading rates. The steady state was maintained for more than one year. Stability process parameters and biogas composition were analyzed twice per week. Nutrients content and COD concentration was measured once per week, while the phytotoxicity was evaluated twice in the whole period, eleven months far from each other.

2.2. Analytical methods for digestate characterization

2.2.1. Physico-chemical analyses

The substrates and the digester effluents were collected and monitored once a week to determine the total and volatile solid content (TS and VS), COD, total Kjeldahl nitrogen (TKN), and total phosphorus (P_{tot}) (American Public Health Association et al., 1999). The process stability parameters, pH, total and partial alkalinity, and ammonia concentration were checked two or three times per week. At steady state conditions, the total polyphenols were analyzed spectrophotometrically using the Folin Ciocalteu assay (Lafka et al., 2007). The concentration was reported in terms of gallic acid equivalent per liter (mg GAE/L). Biogas was collected by a Tedlar® gas sampling bag and the biogas composition (CO_2, CH_4, H_2, and O_2) was determined by a gas chromatograph (GC Agilent Technology 6890 N) equipped with a column HP-PLOT MOLESIEVE, 30 × 0.53 mm ID × 25 mm using a