



Article Co-Composting of Brewers' Spent Grain with Animal Manures and Wheat Straw: Influence of Two Composting Strategies on Compost Quality

Davide Assandri ^{1,2}, Niccolò Pampuro ^{2,*}, Giacomo Zara ¹, Angela Bianco ¹, Eugenio Cavallo ² and Marilena Budroni ¹

- ¹ Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100 Sassari, Italy;
- dassandri@uniss.it (D.A.); gzara@uniss.it (G.Z.); abianco@uniss.it (A.B.); mbudroni@uniss.it (M.B.)
- ² Institute of Sciences and Technologies for Sustainable Energy and Mobility of the National Research Council of Italy, Strada delle Cacce 73, 10135 Torino, Italy; eugenio.cavallo@stems.cnr.it
- * Correspondence: niccolo.pampuro@stems.cnr.it



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: The main challenge of this work is to identify a novel approach to reuse and valorize brewers' spent grain (BSG) to produce a new source of income for the brewers in terms of selfconsumption or selling goods. Therefore, this study explored the composting behavior of BSG mixed with different organic materials: wheat straw with pig slurry solid fraction and wheat straw with sheep manure, MIX1 and MIX2, respectively. The composting process was carried out in bins by comparing two different composting strategies: manual turning (MT) and static composting xxx- without turning operations (ST). During the experimental trial, BSG mixtures were chemically analyzed for pH, total Kjeldahl nitrogen, ammoniacal nitrogen, nitrate-nitrogen, total organic carbon, volatile solids, carbon to nitrogen ratio, and moisture content. Furthermore, the final composted materials were evaluated according to the physicochemical and biological limits fixed by the European regulation (2019/1009) and the Italian law (D.Lgs 75/2010). At the end of the composting process, the C/N ratio ranged from 11.6 to 15.5, the humification ratio ranged from 12.4 to 13.8 and the NH_4^+ -N/NO₃⁻-N ratio was lower than 0.5 indicating, in all investigated treatments, a good degree of maturation. However, as evidenced by the high pH values and low Germination Index, the sheep manure, as starting material, proved less effective than the solid fraction of pig slurry, probably due to excessive trampling and slow litter change. Finally, concerning the two composting strategies investigated, the obtained results highlighted that the composting strategy did not affect the final compost quality.

Keywords: brewers' spent grain; aerobic stabilization; circular economy; recycling; brewing industry; agro-industry by-product; organic fertilizer

1. Introduction

The brewery industry produces large quantities of by-products, typically spent hops, yeast, and spent grain. In particular, brewers' spent grain (BSG) represents 85% of total by-products generated in the brewing process and 20% for a liter of beer produced (20 kg hL⁻¹ beer) [1]. According to the latest Barth report on hops [2], the European beer production, in 2018, was about 531 million hectoliters, while the World production has been estimated at 1904 million hectoliters. Consequently, the worldwide annual production of brewers' spent grain ranged from 38 to 39 million tons [3], with 3.4 million tons in the European Union [4]. Nowadays the main way of recycling BSG is represented by livestock feed production, due to its high content in fiber, un-degradable protein, and water-soluble vitamins [5–8]. However, the high protein content of BSG combined with its high moisture content and fermentable sugar content makes it particularly susceptible to microbial growth and subsequent spoilage over short periods of time, from 7 to 10 days [9]. Several studies report the possibility of reusing BSG as foods for human consumption [10,11], as renewable energy production, in the form of heat, biofuels, ethanol, and biogas [12–14]. Recently many other re-uses have been identified: a substrate for microorganisms and enzymes, antifoaming agents, constituent materials (e.g., biodegradable film, building bricks), paper, absorbent substrates, bio-covers for landfill sites, and a cost-effective sorbent material for wastewater decolorization in the clothing industries [3,15,16]. These recycling methods often require a pre-treatment phase, typically a drying process. However, the drying phase represents an energy-intensive process, which could raise the costs for the breweries [17–21].

The circular economy (CE) business model is defined by technologies that reduce leakages, environmental pollution, input and costs in the production system by implementing Resource Recovery and Reuse (RRR) measures, and recycling waste [22]. Following the above-mentioned principles, composting represents an efficient alternative for organic waste management, allowing the reduction of landfill disposal and, at the same time, recycling its agronomic macronutrients (N, P, and K) content by applying the composted material to agricultural lands [23,24]. Composting is an aerobic process that involves the decomposition of organic matter under controlled temperature, moisture, and oxygen conditions leading to a stabilized final product, free of phytotoxicity and pathogens and with specific humic properties [25,26]. Composting also homogenizes and dehydrates the organic matter enhancing, on the one hand, its uniformity and fertilizing/amending properties and, on the other, increasing the distance that can be run in case of transport for its on-field application.

However, The Circularity gap report (2020) [27] emphasizes that the global economy is currently only 8.6% circular, while in 2018 was 9.1%. Moreover, according to Ellen MacArthur Foundation (2019) [28], less than 2% of the valuable biological nutrients in food by-products and organic waste (e.g., BSG) are composted.

As highlighted by Assandri et al. (2021) [29], the physicochemical characteristics of BSG make this by-product not suitable for direct composting. For this reason, the same authors reported that the addition of lignocellulosic bulking agents (wheat straw, in the specific case) allows the reduction of the moisture content during aerobic stabilization. The addition of this carbon-rich by-product also enhances the optimization of the substrate properties, such as the C/N ratio, air spaces, and pH. Moreover, the addition of livestock manure, as starting material, could be necessary to promote the correct development of the composting process. In this context, Assandri et al. (2021) [29], in their study focused on recycling BSG through the composting process, proposed two different theoretical composting mixtures. These mixtures involved the use of the pig slurry solid fraction and the sheep manure for the co-composting of BSG and wheat straws.

The authors were unable to find any literature on the possibility to recycle BSG through composting process. For this reason, aiming at obtaining an organic fertilizer, a novel approach to reuse and valorize BSG has been investigated. More in detail, the two hypothetical mixtures proposed by Assandri et al. (2021) [29] have been realized and composted in bins comparing two different composting strategies: manual turning (MT) and static composting (ST). The main objective of the study was to identify, for each BSG mixture considered, the best composting strategy in terms of physicochemical and biological characteristics of the final composted material.

2. Materials and Methods

A small-scale composting experiment was carried out, outdoors, at the Institute of Sciences and Technologies for Sustainable Energy and Mobility (STEMS) – Italian National Research Council (CNR) - in Turin, Italy (44°57′ N, 7°36′ E, 245 m above sea level).

The spent grain used in the trial derived from Sommergerstenmalz malt (Pilsner malt) with mashing process by decoction. In 2019, according to the European Statistical Office (2020) [30], pigs and sheep are the most raised animals in Italy with 8.5 and 7.0 million heads, respectively. For this reason, two different types of livestock wastes were investigated as

starting materials: pig slurry solid fraction and sheep manure. The solid fraction of pig slurry (PSF) was obtained from a fattening pig farm after a solid-liquid separation with a screw press (Chior, mod. COM300/600). The sheep manure (SM) was collected in a farm where sheep are raised in paddocks with a slow change in the litter (about 4 times per year). Wheat straw (WS) was used as a bulking agent to optimize the mixture properties such as carbon to nitrogen ratio and air spaces, positively affecting the composting process [24]. The main characteristics of the above-mentioned materials are reported in Table 1.

Table 1. Physicochemical characteristics (mean value and standard deviation of three replicates) of the organic materials involved in the experiment: Wheat Straw—WS; Pig Solid Fraction—PSF; Sheep Manure—SM; brewers' spent grain—BSG. (Dry weight basis).

Parameters	WS		PSI	F	SM	1	BSG	
Turumeters	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Moisture (%) 1	8.0	1.94	66.3	0.97	62.7	1.29	79.6	1.28
pН	5.7	0.06	8.9	0.06	9.3	0.06	4.9	0.75
TOC (%)	55.4	0.30	46.3	0.79	45.8	0.26	55.9	0.05
TKN (%)	0.3	0.03	1.9	0.01	3.3	0.40	4.9	0.28
$NH_4^+-N (mg kg^{-1})$	205	5.03	2120	13.02	2426	25.08	21	1.85
$NO_3^{-}-N (mg kg^{-1})$	154	8.72	32	3.87	81	7.36	10	0.00
VS (%)	95.5	0.51	79.8	1.37	79.0	0.44	96.3	0.08
C/N	207.3	2.41	24.4	0.28	14.0	1.54	11.4	0.67

TOC: Total Organic Carbon; TKN: Total Kjeldahl Nitrogen; NH_4^+ -N: ammoniacal nitrogen; NO_3^- -N: nitrate nitrogen; VS: volatile solids; C/N: Carbon to Nitrogen ratio; ¹ Calculated based on fresh weight.

2.1. Composting Trial and Experimental Conditions

Two composting mixtures (MIX1 and MIX2) were prepared by mixing BSG and WS with PSF and SM for MIX1 and MIX2, respectively. More in detail, MIX1 and MIX2 composition are reported in Table 2.

Table 2. The composition of the two mixtures, MIX1 and MIX2, included in the experiment.

Compost	Weight	BSG	WS	PSF	SM	Total
MIX1	kg	26	8	20	-	54
	percentage	48%	15%	37%	-	100%
MIX2	kg	21	9	-	20	50
	percentage	42%	18%	-	40%	100%

The composting process was carried out in bins by comparing two different composting strategies: manual turning (MT) and static composting – without turning operations (ST). During the 105 days that the composting process lasted, MIX1-MT and MIX2-MT were turned seven times at days 6, 13, 18, 22, 27, 34, and 47.

The composter bins, designed for this trial, were realized following the indications reported by Karnchanawong and Suriyanon (2011) [31] in their study focused on household organic waste composting. More in detail, a total of four 300-L polyethylene bins (base diameter = 600 mm, top diameter = 700 mm, height = 880 mm) were realized for this experiment. Each bin has been equipped with stainless steel grid 100 mm high, with 3 mm holes, to allow air passage and water drainage. Moreover, to drain any leachate formation, each bin was drilled at the base (diameter of 30 mm). Finally, all bins were covered with lids and, as suggested by Karnchanawong and Suriyanon (2011) [31], each lid has been drilled (diameter = 60 mm) in the center, to allow the ventilation.

2.2. Temperature Monitoring

The temperatures from the center of each composting bin were continuously recorded with thermocouples probes (type K) connected to a multichannel acquisition system (Testo, mod 176 T4). Daily mean ambient temperature was also monitored and recorded.

2.3. Compost Sampling and Analytical Measurements

During the experimental trial, three intermediate samplings were performed to investigate the evolution of the mixtures. Each representative sample was obtained by mixing five sub-samples [26]. All composite samples were analyzed for pH, total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N), total organic carbon (TOC), volatile solids (VS), carbon to nitrogen ratio (C/N), and moisture content. Hanna HI 9026 portable pH meter fitted with a glass electrode combined with a thermal automatic compensation system was used to determine the pH in a water-soluble extract 1:10 (w/w) [32]. Kjeldahl standard method [33] was used to determine TKN and NH₄⁺-N content; nitrate nitrogen was evaluated by ion chromatography in a 1:20 water extract [34]. The determination of organic nitrogen took place according to the methods reported by ANPA (2001) and was calculated by the difference between total Kjeldahl-N (TKN) and NH₄⁺-N [35]. Samples for TOC analysis were prepared by drying the samples at 105 °C for 24 h, followed by treatment with sulfuric acid (H_2SO_4) to eliminate any inorganic carbon (C), with subsequent analysis on a C elemental analyzer (EA 1110 Carlo Erba Instruments, Milan, Italy) [32]. The volatile solids content was calculated based on mass loss after heating at 550 °C for 4 h in a muffle furnace [36]. Moisture content was determined after drying the samples in a ventilated oven at 105 °C until a constant weight was achieved; the moisture content was subsequently expressed as a percentage of the raw material [35].

According to Roletto et al. (1985) [37], the determination of the total extractable carbon (TEC) and the humic carbon fraction, consisting of humic CHA and fulvic CFA acids was carried out by oxidative digestion with potassium dichromate and sulfuric acid.

The Humification Ratio was calculated according to the following equation [38]:

$$HR(\%) = (TEC/TOC) \times 100 \tag{1}$$

To characterize the final composts obtained, total Cu and Zn contents were determined and compared with the limits defined by the Italian legislation [39] (D. Lgs 75/2010) for municipal or green waste compost. Total Cu and Zn were analyzed by flame atomic absorption spectroscopy (AA 6800, Shimadzu Corp., Tokyo, Japan) both after sample digestion with concentrated HNO₃/HClO₄ [35,40]. All the analytical determinations were performed in triplicate.

The presence of pathogenic microorganisms in the final composts was estimated by determining the occurrence of *E. coli* and *Salmonella* spp. Enumeration of *Escherichia coli* was performed using cool melted selective culture medium: Tryptone Bile Glucuronide agar (TBX) containing 5-bromo-4-chloro-3-indolyl-ß-D glucuronic acid (BCIG). The number of Colony Forming Unit per gram (CFU g⁻¹) was assessed after incubation at 37 °C for 4 h followed by 44 °C for 21 \pm 3 h [41]. The incidence of Salmonella spp. was determined as described by ISO 6579: 2002 in 25 g of compost samples suspended in 225 mL of peptone water for pre-enrichment at 37 °C for 18 \pm 2 h. The enrichment was performed in Rappaport Vassiliadis broth (RVS broth) and the Muller–Kauffmann tetrathionate/novobiocin broth (MKTTn broth), the samples were incubated respectively at 42 °C and 37 °C for 24 h. Streaks on xylose lysine deoxycholate (XLD) agar and in Salmonella Shigella Agar (SSAgar) were made and incubated at 37 °C for 24 h.

2.4. Germination Index

The phytotoxicity of compost was assessed by evaluating the germination index of cress seeds (*Lepidium sativum*, L.), according to Zucconi et al. (1981) [42]. Compost was mixed with distilled water at a ratio of 1:10 (w/v) and shaken at 150 rpm for 1 h, then centrifuged at 4000 rpm for 20 min and filtered through 0.45 µm Millipore membrane filters. Dilutions were prepared from each sample by adding an equal volume of distilled water (1/1 compost extract/water). Pure distilled water was used as a control. Whatman sterilized filter paper was moistened with five milliliters of extract and placed inside a Petri dish of 9 cm in diameter. Ten seeds were placed on the filter paper and the Petri dishes were incubated in darkness at 25 ± 2 °C. After 48 h of incubation, the percentage of germination and root length were recorded. Three replicates were analyzed for each bin sample. Germination index (GI) was calculated according to the following formula [31,43].

$$GI (\%) = \frac{Seed germination (\%) \times root lengths of treatment (cm)}{Seed germination (\%) \times root lengths of control (cm)} \times 100\%$$
(2)

2.5. Statistical Analyses

The results are presented as mean \pm standard deviation (s.d.). Data of physicochemical parameters were analyzed by a two-way analysis of variance, including in the model Strategy \times Type of the mixture. Differences between means were determined by Bonferroni post-hoc test. Data were previously checked for normal distribution by the Shapiro–Wilk test and homoscedasticity by Levene's test. The referred statistical analyses were performed using R statistic software (R—4.0.3).

3. Results and Discussion

3.1. Temperature Evolution

According to many authors [38,44,45], the temperature pattern shows the microbial activity and the occurrence of the composting process. More in detail, the temperature profile can be used to describe the conditions suitable for the proliferation of thermophilic flora (e.g., *Bacillus* spp., *Thermus* spp., *Thermomonospora* spp.) or mesophilic microbial groups (e.g., *Bacillus* spp. and *Clostridium* spp.) and, therefore, to classify the composting phases [46]. Stentiford (1996) [47] suggested that temperatures higher than 55 °C maximized sanitization, those between 45 °C and 55 °C maximized the biodegradation rates, and between 35 °C and 40 °C maximized microbial diversity in the composting process. Several authors [45,48–52] reported that temperatures above 55 °C for three or five consecutive days are requested for the correct sanitation of the composting mixtures. The temperatures profiles in the four bins and the air temperature, as a function of time, are presented in Figure 1.

During the experimental period, the average daily temperature recorded was 22.6 $^{\circ}$ C, with a measured maximum of 28.9 $^{\circ}$ C and a minimum of 15.5 $^{\circ}$ C.

As shown in Figure 1, between days 1 and 25, the temperature development of MIX1 and MIX2 in MT strategy differed considerably (p < 0.05) from the temperature registered in MIX1 and MIX2 in ST strategy. This variation in the pattern of the temperature is derived from the different turning strategies adopted. In ST, since the turning operation was not performed, at the beginning of the composting process, air circulation was likely to be inhibited and, as a result, the core temperatures decreased faster than in the MT strategy. However, by days 26 until the end of the experiment, the average daily temperatures measured inside MIX1 and MIX2 in MT strategy resulted not significantly different (p > 0.05) than those recorded inside MIX1 and MIX2 in ST strategy.



Figure 1. Average ambient temperature (Ambient) and temperatures development of the two investigated mixtures (MIX1: Brewers' Spent Grain + Wheat Straw + Pig slurry Solid Fraction; MIX2: Brewers' Spent Grain + Wheat Straw + Sheep Manure) with different composting strategies: (MT) manual turning (**a**) and (ST) static composting – without turning operations (**b**). In MT strategy, arrows mark the time of turning.

The temperatures rose quickly in each bin, achieving the thermophilic phase (>45 $^{\circ}$ C) 5–6 h after the filling of the bins (Figure 1). As suggested by Stocks et al. (2002) [53], this rapid temperature rise showed that the brewery by-products, combined with live-stock manure, provide readily available nutrients for the microorganisms involved in the composting process.

In both composting strategies, MIX1 reached the maximum temperature values during the third day: the recorded values in MT and ST strategy were 72.8 °C and 73.0 °C, respectively. Concerning MIX2, in the MT composting strategy, achieved the maximum value (67.2 °C) during the third day while, in the ST strategy, the peak temperature of 64.5 °C was recorded on the second day.

In MT strategy (Figure 1a), the core temperature exceeded 55.0 °C on day 1 and persisted above this level, which sufficed to ensure devitalization of potentially present pathogens, for 8 and 6 days in MIX1 and MIX2, respectively. The temperatures increase observed after each turning operation (Figure 1a) were due to the increased levels of oxygen that stimulate microbial activity [54]. However, after the last turning operation (day 46), the temperatures for both mixtures did not increase, denoting the end of the active phase of the process. As shown in Figure 1a, by days 10 and 22, the core temperatures in MIX2 and MIX1 reached 40.0 °C indicating the end of the thermophilic phase [38].

Concerning ST strategy (Figure 1b), temperatures values above 55 °C were quickly reached and this temperature level persisted for 8 and 4 days in MIX1 and MIX2, respectively. As reported in Figure 1b, the beginning of the mesophilic phase, characterized by temperatures values lower than 40 °C, took place after 12 and 20 days in MIX2 and MIX1, respectively.

3.2. Moisture Content Variation

At the beginning of the composting trial, no significant differences (p > 0.05) were found between MIX1 and MIX2. Specifically, the moisture content values were 73.3% and 73.0% for MIX1 and MIX2, respectively (Figure 2a). Consistent with other published data [29,38,55,56] these values were higher than the optimal range of 60–65% necessary to support microbial activity. This excess of water is probably due to the high moisture content value (79.6%) that characterized BSG (Table 1). However, Liang et al. (2003) [57] reported that maximum microbial activities were provided by the moisture content of about 70%. Moreover, Pampuro et al. (2016) [36] in their study focused on composting pig slurry solid fractions reported similar initial moisture content values. Data reported in Figure 2a show that the moisture level decreased during the process as a consequence of the production of metabolic heat [58]. This result is in line with the study carried out in bin by Zhu (2007) [59] focused on composting swine manure with rice straw. Although the bins remained corked throughout the whole process, in both composting strategies investigated, from the 13th day onwards MIX1 showed significantly (p < 0.05) higher moisture content than MIX2 (Figure 2a). This is probably due to the different physical structures and particle sizes that characterize the two types of manure used. All treatments, except MIX2-ST, showed a slight increase in the moisture content on the 13th day. This increase, as explained by Azim et al. (2018) [60], could be due to the release of metabolic water by microorganisms that break down organic matter in the presence of oxygen, consequently, it could indicate a possible reduction of oxygen in the MIX2-ST treatment.



Figure 2. Changes in chemical properties of the investigated mixtures during the composting process.
(a) Moisture content (%); (b) pH; (c) Volatile solids content (%); (d) Total organic carbon content (%);
(e) Total nitrogen content (%); (f) Ammoniacal nitrogen content (%); (g) Nitric nitrogen content (%);
(h) Carbon to nitrogen ratio. Mean value and standard deviation (vertical bar) of three replicates are shown.

The composting strategy adopted had a significant effect on the moisture content of the final composts. As would be expected, the moisture content values obtained with the MT strategy were significantly (p < 0.05) lower compared to those obtained with the ST strategy. Thus, at the end of the experimental period (day 105), the moisture content values were, in MT strategy, 40.6% and 18.4% for MIX1 and MIX2, respectively, and 46.0% and 26.6% for MIX1 and MIX2 in ST strategy. This difference in moisture content is probably due to the high evaporation rate caused by the turning operations in the MT strategy.

3.3. pH Trend

Changes in pH value depend on the raw materials used for the initial composting mixture and on the conditions of ventilation [60]. Effective ventilation allows a good degradation of the organic material, resulting in a higher final pH. On the other hand, the limited ventilation causes an increase in the content of volatile fatty acids, resulting in a decrease in the pH.

Consistent with other published data [29,38] pH values ranging from 5.5 and 7.5 are considered optimal for microorganisms' development. This factor plays a key role when substrates rich in nitrogen are treated. In this case, high pH values (pH > 8.5), linked to temperatures in the thermophilic range, promote the ammonification with consequently N-losses as ammonia through volatilization [61].

The pH values of the initial mixtures were 8.3 and 8.7 for MIX1 and MIX2, respectively. As shown in Figure 2b, depending on the mixture investigated, the evolution of the pH was different during the process. More in detail, throughout the whole process, MIX2, if compared with MIX1, has been characterized by significantly higher (p < 0.05) pH values.

In MT strategy the values of pH decreased rapidly in MIX1 reaching a final value equal to 6.2 (Figure 2b). In conformity with this trend, Getahun et al. (2012) [54] reported a decreasing pattern of pH during composting of municipal solid waste. Concerning ST strategy, MIX1 followed a similar trend reported by several authors [25,46,62]. After a slight rise (from 8.3 to 8.5) the average pH decreased continuously until the 105th day, reaching a final value equal to 6.0.

Conversely, as shown in Figure 2b, MIX2 maintained alkaline pH values in both composting strategies. From day 1 to day 46, the pH was characterized by a slight upward trend from 8.7 to 9.0 and from 8.7 to 8.8 in MT and ST strategy, respectively. From day 46 to day 74, the pH values dropped slightly, reaching values equal to 8.8 and 8.4 in MT and ST strategy, respectively (Figure 2b). Finally, from day 74 to the end of the composting process there was a significant increase of the pH in both composting strategies investigated reaching a final pH value equal to 9.4 and 9.0 in MT and ST, respectively. This result is in line with the pH values obtained by Storino et al. (2017) [63] after 91 days of active handling followed by 120 days of maturation. As reported by Agapios et al. (2020) [64] and Kumar et al. (2010) [65], the slight increase in pH observed in MIX1 in ST strategy from day 1 to day 13 and in MIX2 in both composting strategies from day 1 to day 46, most likely reflects microbial decomposition of the organic acids and the transformation of the organic nitrogen into ammonium nitrogen. On the other hand, the decline in pH that occurred in MIX1 both in MT and ST strategy could be attributed to the nitrification process which is always accompanied by the liberation of hydrogen ions [66].

3.4. Volatile Solids

At the beginning of the composting trial, there was a significant difference (p < 0.05) in VS content between mixtures. More in detail, the volatile solids content values were 88.6% and 85.5% for MIX1 and MIX2, respectively.

The profiles of volatile solids content during composting are shown in Figure 2c. Consistent with other published data [31,67,68], the volatile solids content, following the degradation rate of the organic matter [69], decrease continuously through the whole composting process.

At the end of the experiment, in ST strategy, no significant differences (p > 0.05) were found between the two investigated mixtures: (77.4% and 77.7% for MIX1 and MIX2, respectively). On the other hand, in the MT strategy, the average VS content value was significant (p < 0.05) higher in MIX1 than in MIX2 (76.9% vs 73.6%).

3.5. Total Organic Carbon

Organic substances are used by microorganisms for their metabolism. This biological degradation, in presence of oxygen, leads to the mineralization of the organic matter in carbon dioxide (CO₂) while, in anaerobic conditions, may result in methane (CH₄) production [70]. Therefore, the TOC content decrease during the composting process.

At the beginning of the trial, the organic carbon content was 51.5% and 48.8% for MIX1 and MIX2, respectively. The statistical analysis showed no significant difference (p > 0.05) between the two investigated mixtures. As expected, due to the degradation of the organic material [54], in both composting strategies and in both composting mixtures, the content of total organic carbon significantly (p < 0.05) decreased with an increase in composting time (Figure 2d). More in detail, the highest carbon loss was observed in MT strategy where, at the end of the composting trial, the TOC content was 42.4% and 42.5% for MIX1 and MIX2, respectively. A decrease in carbon content with increasing turning frequency was also reported by Ahmed et al. (2007) [71], Ogunwande et al. (2008) [72], and Getahun et al. (2012) [54]. Considering ST strategy, the average reduction of TOC content was about 12.7% and 7.5% for MIX1 and MIX2, respectively. These TOC-losses, fall within the same range identified by Guo et al. (2012) [73] in their study carried out in a 60 L stainless steel cylindrical composting reactor.

At the end of the composting process, the two types of mixture showed a TOC content not statistically different (p > 0.05) in their respective composting strategy.

3.6. Nitrogen Dynamics

Typically, if leaching does not occur or is controlled to a minimum, TKN concentration increases during composting as a result of the water loss by evaporation caused by the heat evolved during oxidation of the organic matter [74,75], as well as the net loss of dry mass in terms of carbon dioxide [62].

Consistent with the above-mentioned data, the total nitrogen contents of the four investigated mixtures significantly (p < 0.05) increased after 105 days of composting (Figure 2e). At the beginning of the composting trial, no significant differences were found between MIX1 and MIX2 (2.8% vs 2.6%, p > 0.05). After 105 days of composting (end of the trial), the concentration of TKN resulted significantly (p < 0.05) higher in MIX1 than in MIX2. Specifically, MIX1 and MIX2 reached TKN values equal to 3.7% and 3.1%, and 3.5% and 2.9% in the MT and ST strategies, respectively.

Considering the two composting strategies investigated, no significant difference (p > 0.05) was observed between MT and ST. This result is in contrast with the study conducted by Brito et al. (2008) [56], which focused on composting of the solid fraction of dairy cattle slurry, where the authors highlighted that turning operation if compared with a static pile, increase the N concentration.

Nitrogen transformations in the first stage of composting of nitrogen-rich material are generally characterized by high rates of ammonification as a consequence of ammonifying activities resulting from the development of microbial activities. The greatest nitrogen losses during composting are typically caused by ammonia emissions.

The two types of manure utilized were characterized by a high initial NH₄⁺-N content (Table 1). In particular, at the beginning of the composting process (day 0), MIX1, if compared with MIX2, showed the lower initial concentration of NH₄⁺-N (1091 mg kg⁻¹ vs 1623 mg kg⁻¹; p < 0.05). This difference is probably due to the different NH₄⁺-N concentrations characterizing PSF and SM (Table 1).

Consistent with other published data [56,76,77], after 13 days the NH₄⁺-N content decreased in all the investigated treatments (Figure 2f). The highest NH₄⁺-N concentrations were associated with static composting. More in detail, at the end of the composting process (day 105), NH₄⁺-N values were equal to 187 mg kg⁻¹ and 281 mg kg⁻¹ for MIX1 and MIX2 in MT strategy and 247 mg kg⁻¹ and 408 mg kg⁻¹ for MIX1 and MIX2 in ST strategy. This significant difference (p < 0.05) between MT and ST is probably due to reduced ammonia volatilization as a consequence of the lack of ventilation in the ST strategy [78].

The nitrification process, promoted by nitrifying bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.), leads to the formation of NO_3^- -N when the temperature of the mixture is below 45 °C. However little nitrification may occur also under thermophilic conditions [62].

In our study, there was no nitrification during the thermophilic stage since the nitrification bacteria are limited by temperatures above 45 °C from day 0 to day 20 (Figure 1) and high-temperature competition for oxygen (for aerobic respiration) was so hard that nitrification was not possible (Figure 2g). This is in agreement with the study carried out by Venglovsky et al. (2005) [79] focused on pig slurry solid fraction composting.

The initial nitrates content of the two mixtures was significantly different (p < 0.05), precisely 74 mg kg⁻¹ for MIX1 and 103 mg kg⁻¹ for MIX2. As previously reported considering NH₄⁺-N content, this difference is probably due to the different NO₃⁻-N concentrations characterizing PSF and SM (Table 1). As would be expected, at the end of the trial, the nitrate values obtained without turning operations were higher (p < 0.05) compared to those obtained in the MT strategy. In particular, the final nitrate values were equal to 490 mg kg⁻¹ and 560 mg kg⁻¹ for MIX1 and MIX2 in MT strategy and 836 mg kg⁻¹ and 931 mg kg⁻¹ for MIX1 and MIX2 in ST strategy. This trend is in line with the pattern described by Brito et al. (2008) [56] in their study focused on composting the solid fraction of dairy cattle slurry.

3.7. Carbon to Nitrogen Ratio

The carbon to nitrogen ratio plays a key role in defining the nutritional balance of a composting mixture. More in detail, carbon, and nitrogen are required by microorganisms, as an energy source, for their development and activity. The optimum C/N ratio for composting is in the range 20–35 [38,44,61,70,80,81]. Composting mixtures characterized by C/N ratio >35 make the process very slow due to the excess of degradable substrate for the microorganisms, while C/N ratio < 20 can result in nitrogen losses, as ammonia volatilization or as leachate from the composting mass, due to the excess of N per degradable C [82].

At the beginning of the composting process MIX2 was characterized by carbon to nitrogen ratio significantly (p < 0.05) higher than MIX1 (19.1 vs 18.2). Both mixtures presented a C/N ratio lower than the optimum level established. However, the C/N values obtained in the present study are close to those reported by Chiumenti et al. (2007) [55] in their trial focused on composting pig manure and by Tabrika et al. (2020) [83] in their experiment focused on composting tomato plants residues with sheep manure.

As shown in Figure 2h, in all investigated mixtures, with an increase of composting time, there was a decrease in the C/N ratio. MIX1 decreased from 18.1 to 11.6 and 13.0 for MT and ST strategy, respectively. MIX2 decreased from 19.1 to 13.9 and 15.5 for MT and ST strategy. The C/N ratio decreased mainly due to the conversion of organic carbon into carbon dioxide [84], and also to the increase in total nitrogen content in the final composted material [85].

The carbon to nitrogen ratio was also significantly (p < 0.05) affected by the turning operations. At the end of the composting process, the highest C/N ratio was observed for MIX2-ST (15.5), while the lowest C/N ratio was observed in MIX1-MT (11.6). This result is in line with the study conducted by Getahun et al. (2012) [54] focused on composting municipal solid waste.

3.8. Maturity of the Final Composts

Compost derived from BSG, representing a large reservoir of nutrients, can be re-used as soil fertilizer and conditioner, to replace the more expensive and less environmentally sustainable chemical fertilizers for crop production. However, immature compost, generating adverse effects on plant growth and/or seeds germination, limiting the agricultural application of this organic product [81,86,87].

To assess compost maturity, chemical methods, including C/N ratio [88], nitrification (NH_4^+-N/NO_3^-N) [89], and humification ratio [38], are typically used.

Consistent with published data [88–92], the C/N ratio below 20 is indicative of mature compost. As previously reported, at the end of the composting trial, the C/N ratio was equal to 11.6, 13.0, 13.9, and 15.5 for MIX1-MT, MIX1-ST, MIX2-MT, and MIX2-ST, respectively.

The oxidation of ammonium to nitrate, operated by nitrifying microorganisms, occurs mainly during the mesophilic phase, and is completed during the maturation phase [83]. For this reason, the NH_4^+ -N to NO_3^- -N ratio has also been used to estimate compost stability and maturity [88]. Generally, NH_4^+ -N/ NO_3^- -N value lower than 1.0, is considered indicative of mature compost [78,85,88]. In our study, this ratio decreased during the composting process and, in all investigated treatments, fell below 0.50 after 105 days of composting indicating a good degree of compost maturation (data not shown).

The agronomic value of composted material is closely linked to the humification degree. During the composting process, due to microbial degradation, humic substances are produced and humic acid-like organic-C (CHA) increases, while fulvic acid-like organic C (CFA) decreases [83,90]. At the end of the composting trial, MIX1 achieved HR equal to 13.8 and 13.6 in MT and ST strategies, respectively while MIX2 was characterized by HR equal to 12.4 and 12.6 for the respective strategies. According to Bernal et al. (2009) [38] an HR higher than 7 indicates a good humification degree.

The germination index, which combines the measure of relative seed germination and relative root elongation of cress seed, is an integrated biological indicator, which is regarded as the most sensitive parameter used to evaluate the toxicity and degree of maturity of compost [42]. At the end of the composting process, a GI value equal to 95%, 37%, 93%, and 65% was recorded for MIX1-MT, MIX2-MT, MIX1-ST, and MIX2-ST, respectively. Zucconi et al. (1981) [42] reported that GI value above 80% indicated the disappearance of phytotoxicity in compost. Thus, concerning MIX1, 105 days were sufficient to overcome the threshold limit of 80%. On the other hand, MIX2, being characterized by GI values lower than 80% could negatively affect plant growth and seed germination.

3.9. European and Italian Law Limits

The characteristics of the final composts in comparison with the quality parameters required by the European regulation (2019/1009) [93] and Italian law [39] (D.Lgs. 75/2010) are reported in Table 3. All parameters respected the fixed limits, with the exception of the pH in MIX2 and the GI value in MIX2-MT. The Italian law evaluates the stability of the OM of the compost taking into consideration the C/N ratio, the total organic carbon content (TOC), and the sum of humic acid-like carbon (CHA) and fulvic acid-like carbon (FCA), which must be less than 25, greater than 20% and greater than 7%, respectively. Moreover, in relation to nitrogen, the only parameter considered is the total organic nitrogen content, which must be, at least, 80% of the TKN. Instead, the recent European regulation highlights that humidity and TOC must be less than 80% and at least 7.5% by mass, respectively. Concerning copper and zinc contents, the four investigated composts were characterized by values lower than the fixed limit (Table 3). In our study, only Cu and Zn contents were investigated because it is well known that slurry and other manure from intensive animal farming are typically characterized by a high concentration of these two heavy metals. The high concentration of these two metals in manures derives, especially for pigs, from the traditional practice of adding copper and zinc salts as feed additives, to ensure good performance, increasing feed efficiency and productivity. However, only a small proportion

(5–10%) of dietary Cu and Zn is absorbed by the pigs, while the rest is voided in the pigs' feces [26].

Finally, microbiological analyses allowed verifying the effectiveness of the process in terms of the destruction of pathogens accomplished by the heat produced during the thermophilic phase. *Salmonella* spp. and *E. coli* were absent in the final composted materials of all the investigated mixtures (Table 3). This is in line with the limit fixed by European regulation and Italian law.

Table 3. Physicochemical and biological characteristics of the final composts, with the respective Italian law (D.Lgs. 75/2010) and European Regulation (2019/1009) limits.

Parameters	Unit	МТ			ST				Italian Law	EU Regulation	
		MIX1	s.d.	MIX2	s.d.	MIX1	s.d.	MIX2	s.d.	Limits	Limits
pН		6.2	0.06	9.4	0.00	6.0	0.12	9.0	0.00	6.0 - 8.5	-
Moisture ¹	%	40.6	0.63	18.4	0.73	46.0	1.76	26.6	0.86	<50%	<80%
TOC	%	42.4	0.13	42.5	0.95	44.9	0.03	45.1	1.15	>20%	>7.5%
Total organic N	%	3.5	0.06	2.8	0.21	3.1	0.15	2.8	0.06	>80% of TKN	-
C/N		11.6	0.29	13.9	0.28	12.9	0.64	15.5	0.21	<25	-
CHA + FCA	%	13.6	0.25	15.8	0.72	11.9	0.14	15.9	0.32	>7%	-
Total Zn	$ m mgkg^{-1}$	479	2.76	202	2.40	419	1.69	228	1.16	$<500 \text{ mg kg}^{-1}$	$<\!800 { m mg kg^{-1}}$
Total Cu	$mg kg^{-1}$	73	0.50	24	3.36	68	3.04	30	0.76	$<150 \text{ mg kg}^{-1}$	$<300 \text{ mg kg}^{-1}$
GI (30%)	%	95	1.00	37	2.52	93	5.69	65	5.86	>60%	-
Salmonella spp.	$CFU 25g^{-1}$	abs	-	abs	-	abs	-	abs	-	absent in 25 g	absent in 25 g
E. coli	$CFU g^{-1}$	abs	-	abs	-	abs	-	abs	-	$< 1000 \text{ CFU g}^{-1}$	$< 1000 \text{ CFU g}^{-1}$

CHA + FCA, humic carbon fraction; GI, germination index; ¹ Calculated based on fresh weight.

4. Practical Implications of This Study

The main challenge of this work is to identify a novel approach to reuse and valorize brewers' spent grain to produce a new source of income for the brewers in terms of selfconsumption or selling goods. Particularly, in a circular economy model, composted BSG can be exploited for agronomical purposes for the cultivation of raw materials such as cereals, hop, or any flavoring vegetal essence. This process should be favored by local policies through the constitution of enterprises' networks and the creation of a short supply-chain district. However, the high moisture content of BSG represents one of the major concerns for the composting process. Therefore, the addition of bulking agents, such as wheat straw, to reduce the moisture content during the process is mandatory. Moreover, the addition of livestock manure is fundamental for the correct development of the composting process.

Aiming at evaluating the proposed composting strategies in terms of environmental impact, further research activities will be carried out. More in detail, an experimental trial focused on GHG monitoring will be planned.

5. Conclusions

The most important conclusion is the feasibility of recycling the BSGs through the composting process, thus giving the agro-breweries the possibility of self-producing a soil improver, reducing the spaces for storing BSG, and limiting pollution due to the high moisture content of this by-product. According to the data provided above, all the composting mixtures investigated had reached an acceptable degree of maturation at the end of the process. However, as evidenced by the high pH values and low GI values, the sheep manure (MIX2), as starting material, proved less effective than the solid fraction of pig slurry, probably due to excessive trampling and slow litter change. Finally, concerning the two composting strategies investigated—manual turning and static

composting—the obtained results highlighted that composting strategy did not affect the final compost quality.

Despite the small size of the compost piles, thanks to this study it is possible to have guidelines on the composting of BSG. However, to have the certainty of the real possibility of co-composting BSG, it would be desirable an experimental design with several tons of initial mixtures.

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