Bokashi

– kitchen waste treatment without greenhouse gas emissions?

Bokashi- köksavfallshantering utan växthusgaser?

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Abstract

EM Bokashi is a fermentative treatment used for kitchen waste, branded as a climate friendly alternative to compost with low or no greenhouse gas (GHG) emissions. This study aims to examine if these claims are true. This was done by examining if adding of Effective Microorganisms (EM) reduces GHG emissions (CO_2, CH_4 and N_2O) during the fermentation phase of Bokashi. Additionally, a literature review over EM Bokashi was made to compare with household composts emissions.

The experimental setup was three lines with four replicas, comparing adding of EM, adding of autoclaved EM and a blank with only kitchen waste added. Bokashi buckets were installed with gas ports in the lids for vial sampling. Samples was analysed for CO_2, CH_4 and N_2O concentrations with gas chromatography. Additionally, C and N content, weight loss and moisture were measured together with leachate pH.

The results show no significant difference between the treatments in total emissions. However, a trend for lower N_2O emissions with EM Bokashi treatment was observed, and during the resting phase CH_4 was significantly lower compared both to the Autoclaved and Control treatment. The review gave insufficient information to draw any conclusions of GHG emissions compared to compost. The fermentation phase release relatively low CH_4 emissions, but the last step of Bokashi, (*the soil factory step)* have not been studied. That step is where the main degradation occurs, and therefor theoretically the step with the highest risk for GHG emissions. CO_2 emissions seems to be similar for EM Bokashi and compost.

Keywords: EM, Bokashi, GHG emissions, Greenhouse gas, CO_2, CH_4, N_2O

Populärvetenskaplig sammanfattning

EM Bokashi är en alternativ metod till kompost, som uppfanns av Teruo Higa på 80-talet. Intresset för metoden har ökat i Sverige på senare tid och säljs nu som ett färdigt koncept för odlingsintresserade hushåll. I korthet går det ut på att syra köksavfall i lufttäta behållare, för att sedan gräva ner det i jord och låta Bokashins organiska material brytas ner. Processerna sätts igång genom att tillsätta så kallade Effektiva mikroorganismer (EM). I svensktillverkat bokashiströ används en jästsvamp, två mjölksyrabakterier, och två proteobakterier. Jästsvampen och mjölksyrabakterierna är till för att sätta igång syrningsprocessen, medan proteobakteriernas huvudsakliga funktion ska vara att fixera kol genom fotosyntes.

EM Bokashi marknadsförs med påståendet att den släpper ut väldigt låga halter växthusgaser jämfört med kompost som påstås släppa ut höga halter. Ibland påstås det till och med att ingen koldioxid eller metan släpps ut. Det här arbetet gick därför ut på att ta reda på vad påståendena har för vetenskapligt stöd. Det gjordes dels genom en litteraturundersökning, dels genom att testa om växthusgasavgången under syrningsprocessen minskar vid tillsats av EM. I introduktionen tas också typiska nivåer av växthusgasavgång från hemkomposter upp tillsammans med olika riskfaktorer, för att kunna sätta I relation till litteraturundersökningen.

I experimentet mättes koldioxid, metan och lustgas. Ingen signifikant skillnad hittas i total avgång mellan behandlingarna att tillsätta EM, att tillsätta avdödad EM och att bara fylla på med köksavfall. Det fanns däremot en trend som visade på lägre lustgasutsläpp i behandlingen med EM. Efter att behållarna var fyllda och matavfallet fick syras vidare med stängda lock var både metan och lustgasavgången lägre, medan koldioxidavgången var något högre med EM.

Litteraturundersökningen kom fram till att det finns ett bristfälligt stöd för påståendet att EM Bokashi släpper ut mindre växthusgaser. Till att börja med hittades få artiklar som undersökt frågan. Den artikel som Bokashi i Sverige AB hänvisar till på sin hemsida kunde inte hittas i publicerad version och undersöker bara syrningsfasen. En artikel hittades där EM testats som tillsats för produktion av biogas (koldioxid och metan). Den kunde inte mäta någon metanproduktion. Lustgas spekulerades det egentligen bara kring i en artikel, som såg en förhöjd risk om köksavfallet innehåller mycket kväve. Koldioxid bildades i studien över biogasproduktion. Två studier konstaterade en ökad markandning när EM bokashi hade tillförts till jord. En systerstudie till den här kom också fram till att EM Bokashi avgav mer koldioxid än kompost efter att den blandats med jord.

De nivåer som släpptes ut under syrningsfasen var mycket lägre än de värden som hittats för hela kompostprocessen. Vad man ska komma ihåg är dock att de var lägre oavsett om EM var tillsatt eller inte. Det är alltså troligt att andra faktorer var viktigare i experimentet såsom en en låg C/N kvot i köksresterna. Syrning är också en konserveringsmetod vilket gör att den huvudsakliga nedbrytningen troligen sker efter det. Att markandningen ökar kortsiktigt kan innebära en ökad risk för växthusgaserna metan och lustgas. Samtidigt hittades inga mätningar av de gaserna över senare

delen av bokashiprocessen. Det finns också kunskapsluckor i hur EM interagerar med varandra och den mikroflora som redan finns i jorden.

Det finns ingen vetenskaplig konsensus kring att EM Bokashi släpper ut mindre växthusgaser eller att mer kol binds till marken. Att tillsätta EM gav en reducerande effekt på metanutsläpp efter att syrningsprocessen kommit igång ordentligt och locket hålls stängt. Något som också bekräftas av en studie över EMs potential för biogasproduktion. Koldioxidavgången var istället högre med tillsatt EM när syrningen var igång. Och efter att ha blandat med jord visade flera studier på en ökad koldioxidavgång. Systerstudien visade att den totala kolavgången jämnas ut i och med det. Inga tidigare mätningar av lustgas har hittats. Här fanns en trend mot lägre avgång i total mängd över matning och syrning men ingen signifikant skillnad fanns. Det finns alltså kunskapsluckor där också.

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Abbreviations

AC	Autoclaved treatment
C	Control
ECD	Electron Capture Detection
EM	EM Bokashi (Effective microorganism)
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization of the United Nations
FID	Flame Ionization Detection
GC	Gas Chromatography
GHG	Greenhouse Gas
GWP	Global Warming Potential
IPCC	Intergovernmental Panel on Climate Change
SLU	Swedish University of Agricultural Sciences
WW	Wet waste

1. Introduction

1.1. Aim and background

EM Bokashi is advertised as an environmentally friendly alternative to regular kitchen compost, with less leaching of nutrients and no greenhouse gas (GHG) emissions. In printed manuals and folders *Bokashiworld in Sweden AB* (2018) state that EM Bokashi does not produce carbon dioxide (CO_2) nor methane (CH_4) and on their official Swedish blog they claim that compost produce large amounts of GHGs while Bokashi produces very small amounts (Harlen 2015). The claim that Bokashi releases low or even no GHGs have now been quoted in different contexts from gardening literature (Peter Streijffert 2019), to televisions shows (*Mandelmanns gård,* 2020), and newspapers (Ekstrand 2018). However, it is questionable if these claims rest on a scientific foundation. This thesis therefore aims to evaluate if the adding of EM Bokashi reduces GHG emissions. Both a review and a lab experiment will be performed to test this.

In this study Bokashi refers to the method of adding Effective Microorganisms (EM) to kitchen waste in an airtight bucket. Instead of composting, the waste is fermented by the added microorganisms in the microaerobic environment created.

1.2. Introduction

1.2.1. Food waste and organic waste

According to FAO (2018) 1.3 billion tonnes of food produced for human consumption will end up as food loss and waste annually. This is equivalent to one third of the total amount produced. In medium and high-income parts of the world (North America, Europe) the source of loss and waste happens mainly in the distribution and consumption line and is equivalent to 280-300 kg/cap annually. In Sweden, the food waste produced reached 1.3 million tonnes 2018, where the part

from households alone was 917 000 tonnes or 95 kg/cap according to the Swedish EPA report *Matavfall i Sverige* (2018).

To quantify the global GHG emissions from kitchen waste can be difficult since the terms *food loss* and *food waste* refers to emissions from the whole life cycle of food production. From the waste sector perspective, the terms *organic waste* or *bio-waste* is more commonly used. EU uses bio-waste in their directives, but apart from kitchen and food waste (from households, restaurants, caterers and retailers) similar waste from food processing plants as well as garden and park waste are also included.

1.2.2. Composting to reduce GHG emission of organic waste

In the report *AR4 Climate Change 2007: Mitigation of Climate Change — IPCC* it is mentioned that the whole waste sector stands for less than 5% of the global anthropogenic GHG emissions. In the EU the waste section is the fourth biggest emitter of GHG emissions. In IPCC modelling set to reach the 1.5-degree goal, a reduction of CH_4 and black carbon (C) emissions of 35% respectively between 2010-2050 is accounted for. Methane emissions from the waste sector is mentioned as a major source. As a mitigation strategy, organic waste is increasingly recycled through composting or anaerobic digestion instead of being dumped in landfills. One important aspect of this recycling is that it can replace peat as growing media and the production mineral fertilisers (Andersen et al. 2012).

Composting is a mitigation strategy compared to landfills but still results in GHG emissions. This is evident from an increasing trend of emission from biological waste treatment in Sweden. The Swedish EPA explicitly mention increasing composting and anaerobic digestion as the reason for this and quantified it to 130 000 tonnes CO_2-equivalents for 2016 (Swedish EPA, 2016).

1.2.3. GHG from kitchen composting

Even though composting is only a minor contributor to global GHG emissions (*IPCC*, 2007), there is a risk of CH_4 and nitrous oxide (N_2O) being produced. These are GHGs with the global warming potential (GWP) of 28-34 and 265-298, respectively, over a period of 100 years (*IPCC 2013*). GWP is based on the radiative forcing resulting from an emission pulse of a GHG, in relation to radiative forcing of the same mass of CO_2. The higher values of the GWP include Climate carbon

feedback, which take into consideration how the carbon cycle is affected by additional C in the atmosphere (Gasser et al. 2016), and are therefore used in this thesis.

Ideally composting will only result in aerobic respiration, but there is always anaerobic conditions or semi aerobic conditions in parts of a compost pile. The reason for the anaerobic conditions is that the microorganisms consume O_2 faster than additional O_2 is diffused to micro areas. A high decomposition rate together with compaction and a high-water content are underlying factors (Paul 2015). N_2O and CH_4 production in compost piles is reduced by aeration and adjustment of water content, C/N ratios and void space (Yang et al. 2013).

The reduction of GHGs from composts has been scientifically studied for decades, but not specifically home composting (Andersen et al. 2010). Articles from the latest decade have started to address home composting (Table 1). The aim is often to better understand different parameters that can reduce the risk of CH_4 and N_2O emissions. In the ranges presented in Table 1, control treatments are included and are in general the higher values. That means that controlling of different management and parameters might help keeping emissions from home composts on a lower level.

Table 1: N_2O and CH_4 emissions from home or kitchen composting. The emission range represents different treatments and control treatments.

N_2O kg/t ww	CH_4 kg/t ww	Organic waste/method	Subject	Source
0.30-0.71	0.60-1.65	Home composting	Biofilters	(Amlinger et al. 2008)*
0.30-0.55	0.4-4.2	Household waste and small amount of garden waste	Mixing	(Andersen et al. 2010)
<0.0014-0.05	0.55-1.56	Kitchen waste with different bulk agencies	Bulk agent	(Yang et al. 2013)
0.12-0.14	0.06-0.44	Kitchen waste with phosphogypsum/superphosphate	Adding phosphogypsum and superphosphate	(Yang et al. 2015)
0.0037-0.016	0.0097-6.7	Food and garden waste	Moisture content	(Ermolaev et al. 2019)

**Information taken from 2ndary source (Boldrin* et al. *2009)*

As mentioned by Ermolaev *et al.* (2014), home composting differs from large scale systems both in management and process parameters. Their study showed a lower CH_4 production from home composting compared to large scale (comparing CH_4:CO_2 ratios), which can be explained by easier aeration in a small pile. Connected to aeration of home composting, both Andersen *et al.* (2010) and Ermolaev *et al.* (2014) surprisingly showed that turning of the compost material increases CH_4 emissions. In the study of Andersen *et al.* (2010), the CH_4 and N_2O

emissions varied between 0.4 and 4.2kg/t and 0.30-0.55kg/t input of wet waste, respectively. The most frequently turned compost developed the greatest amount of CH_4 and the ones that were not turned the least. For Ermolaev *et al.* (2014) the mean CH_4 emission was 28.1 ppm and N_2O 5.46 ppm(v/v) above ambient air samples, with a CH_4:CO_2 ratio of 0.38 and N_2O:CO_2 ratio of 0.15. Moisture and increased temperatures also gave higher CH_4 emissions.

In a more recent study, Ermolaev *et al.* (2019) could show that an increase of moisture between 44 % and 66 % led to an exponential increase of CH_4 emissions from 0.04 to 35 g kg^{-1} initial C. N_2O emission was lowest for 59 % moisture (0.3 g kg^{-1} initial N) and highest for 66 % moisture (1.43 g kg^{-1} initial N).

No consensus concerning which unit to use when estimating GHG emissions from compost seems to exist, which makes further comparisons more challenging. Some examples of units are CH_4/kg wet waste, CH_4/kg dry waste, g CH_4-C/kg input C wet waste, ppm (v/v) above ambient air, ratios between gas and carbon dioxide (CH_4:CO_2).

1.2.4. Microbial metabolism pathways: aerobic respiration, anaerobic digestion, and fermentation

Composting and anaerobic digestions are the two most common ways of recycling organic waste. For EM Bokashi, the waste is fermented instead. To better understand the risk of GHG emissions during these processes, a general overview of the metabolic pathways in these processes are helpful.

Composting: Aerobic respiration

Composting is a controlled rapid degradation of organic matter in an aerobic environment. It results in a nutrient rich substance with stable organic content (Harrison 2008). Composting can be divided into four stages: the mesophilic, thermophilic (>40°C), cooling and maturation phase. In the first two steps labile C is rapidly decomposition. This process generates heat which eliminates many pathogens. The microbial community changes with the stages, adapted after different temperatures and substrate (Smith et al. 2015).

Ideally composting will only result in aerobic respiration, where O_2 is used as the final electron acceptor which is being reduced to water, and organic C is oxidised to CO_2. However, there is always anaerobic conditions or semi aerobic conditions in parts of a compost pile. The reason for the anaerobic conditions is that the aerobic respiration consume oxygen (O_2) faster than additional O_2 is diffused to micro areas. A high decomposition rate together with compaction and a high-water content are underlying factors creating anaerobic niches (Paul 2015). CH_4 and N_2O

production in compost piles is reduced by aeration and adjustment of water content, C/N ratios and void space (Yang et al. 2013).

Risk of CH_4 emissions

During anaerobic conditions microbes that uses other final electron acceptors than O_2 to gain energy can thrive. Anaerobic digestion is a four-step process including hydrolysis, acidogenesis, acetogenesis and methanogenesis. GHGs produced in these processes are CH_4 and CO_2. During methanogenesis CO_2 is reduced to CH_4, while a reduced organic compound (such as acetate) act as electron donor being oxidised to CO_2 (Paul 2015). Methanogens performing this are strictly anaerobic archaea.

Risk of N_2O emissions

N_2O is produced from nitrification and denitrification (Butterbach-Bahl et al. 2013; Ermolaev et al. 2014). Nitrification is mainly an aerobic process while denitrification is anaerobic (Paul 2015). The main driving force for nitrification is the amount of available ammonia (NH_4^+). This is connected to a high rate of decomposition and mineralisation (Paul 2015). Kitchen waste is rich in N and therefore a substrate likely for this to occur. A rule of thumb is that substrate with lower C/N ratios than 25:1 results in mineralisation of N (Paul 2015). pH can also affect available NH_4^+ level, where a lower pH reduce availability by pushing the equilibrium $NH_4^+ \leftrightarrow NH_3 + H^+$ to the left (Su et al. 2019).

Denitrification is the main source of N_2O emission globally. Denitrifies are often facultative anaerobes that uses nitrate (NO_3^-) as a final electron acceptor when O_2 is limited. Commonly they are heterotrophs using reduced carbon as an electron donor. Nitrification and denitrification are connected since the former produces and the latter consumes nitrate. The rate of nitrification can therefore limit denitrification. If the O_2 levels fluctuate in a way that nitrification can occur without consuming all carbon, perfect conditions for denitrification is achieved (Paul 2015).

EM Bokashi: Fermentation

The concept of EM Bokashi is to add an inoculum of microorganisms to kitchen waste that will result in lactic acid fermentation. This is an anaerobic process that generates relatively low amount of energy for the organisms and is therefore not desirable for them in aerobic conditions. It is a shorter pathway than anaerobic digestion. After the glycolysis, pyruvate is transformed into either ethanol or lactic acid. Both ethanol and lactic acid can outcompete other organisms, since the first is toxic to many and the second lower the pH. Normally lactic acid fermentation is used as a preservation method.

Instead of using aeration the idea with Bokashi is to prevent GHG emissions by lowering the pH. This prevents degradation to occur before mixing the matured EM Bokashi with soil. Methanogens in general prefer a pH between 6 and 8 (Kim et al. 2013). A lower pH has been showed to inhibit CH_4 production in studies of biogas production in food waste. For example, one study that examined the pH-range 5-9 showed that 7 was optimal and conditions with pH 5 produced the least CH_4 (Widya Rani et al. 2018).

However, the risk of CH_4 and N_2O is still implied. If the fermentation fails (e.g., batch with less active EM) anaerobic digestion can produce large amounts of CH_4. Also, fermentation is only the first step of Bokashi. Afterwards the fermented product is covered with soil for further degradation (the soil factory step), before using as a fertiliser.

1.2.5. Traditional and EM Bokashi

Bokashi is a Japanese word that is translated “to blur” “to gradate” or “shading of” in several dictionaries (*Japanese Dictionary Tangorin*; *RomajiDesu; Japanese dictionary*). According to Nishio (1996) it refers to a traditional Japanese composting method, where organic fertilizer is partly composted to prevent pest. The compost is inoculated with microorganisms and water is added to a 50-55% moisture content. The compost pile is mixed several times during the process to prevent higher temperatures. As a last step the compost is dried and packed into bags.

The subject of this thesis refers to EM Bokashi, a concept developed by professor Teruo Higa in the 1980’s (*EMRO, 2020*). This differs from traditional bokashi in several ways. Instead of mixing the organic waste it is placed in a closed container and inoculated with EM. The waste is compressed to prevent aeration. When the container is filled, it is left to be fermented for at least 2 weeks. As a second step the fermented product is buried in soil for further degradation (*Bokashiworld in Sweden AB,* 2018).

The EM inoculum contains a combination of at least five species of microorganisms (*Justia Patents Search, 1997*). *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*, *Rhodopseudomonas palustris* and *Rhodospirillum rubrum* are mentioned on the Swedish retailers official website (Harlen, 2018).

Lactobacillus plantarum are facultative heterolactic bacteria, meaning they can either perform homolactic fermentation, or heterolactic fermentation (Larimer et al. 2004). In homolactic fermentation glucose is transformed to lactic acid and in

heterolactic fermentation the end-products are ethanol, lactate, acetate and CO_2 (Ciani et al. 2013). Sequencing of the genome have shown enzymes that produce formate, acetoin, ethanol, acetone and 2,3-butanediol support the heterolactic pathway (Larimer et al. 2004).

Lactobacillus casei are also facultative heterolactic bacteria (Ibrahim 2016). Both *L. plantarum* and *L. casei* have genes that indicate that they are relatively tolerant compared to other in the same family and can use aerobic respiration (Zotta et al. 2017).

Saccharomyces cerevisiae is a yeast fungus. It is facultative anaerobic, with fermentation end products CO_2 and ethanol (Deák 2003). *S. cerevisiae* is tolerant to low pH and high ethanol concentrations (Nevoigt 2008). Bokashiworld in Sweden AB highlights that this yeast function as a stimulation of bacterial growth of lactic acid bacteria (Harlen, 2018).

Rhodopseudomonas palustris can use four different types of metabolisms: photoautotrophic, photoheterotrophic, chemoautotrophic and chemoheterotrophic (Larimer et al. 2004). This makes them flexible in terms of energy in carbon source.

Rhodospirillum rubrum are proteobacteria that can use photosynthesis under anaerobic conditions, but also grow in dark under aerobic conditions (Gest 1951 see Selão 2010). In Bokashiworld in Sweden AB's description of *R. palustris* and *R. rubrum* they focus on their carbon fixation ability (Harlen, 2018). For *R. rubrum* these are light dependent functions, and not relevant during the fermentation process. When exposed to sun the anaerobic condition is compromised instead.

1.2.6. Hypothesis

With this background the hypothesis is that 1. GHG emissions from Bokashi and compost are similar, 2. the adding of EM bokashi to an anaerobic environment will decrease production of GHGs, but 3. it will however release CO_2 from respiration and some amount of CH_4 and N_2O.

2. Material and Methods

2.1. Literature review

A literature survey over previous studies concerning Bokashi and GHG emissions was performed in PRIMO and Web of Science using the search words: “Bokashi”+“GHG”; “Bokashi”+”greenhouse gas*”; “Bokashi”+”methane”; “Bokashi”+”CH4”; “Bokashi”+”Nitrous oxide”; “Bokashi”+”N2O”; “Bokashi”+”carbon dioxide” and “Bokashi+CO2”.

2.2. Experimental design

2.2.1. Setup

The experiment consisted of three series, with four replicas each. The first was treated with EM Bokashi bran, the second was treated with autoclaved EM Bokashi bran, and the third was a control of kitchen waste only. These treatments will be referred to as EM Bokashi/EM, Autoclaved/AC and Control/C treatment.

Figure 1: Bokashi bucket installed with gas sampling devices.

A total of twelve 16 L plastic airtight Bokashi buckets with strainers and taps, provided by Bokashiworld in Sweden AB, were used as containers. To facilitate gas sampling, two holes were drilled in the lid, where valves were installed and connected to 10 cm plastic tubes on the inside and outside (Figure 1). The tubes on the outside were connected to a plastic fitting with a needle and a steel adapter (female part) for being connected to a pump and vial for GHG measurements. The tubes on the inside were cut as the height of the kitchen waste increased. One was removed to create better mixing.

The lab experiment was performed between Feb-11 and Mar-24 2020, divided into a feeding phase and a resting phase. During the feeding phase kitchen waste + treatment was added to the 12 buckets, and in the resting phase the bokashi matured with the lid kept closed. The schedule can be seen in Figure 2.

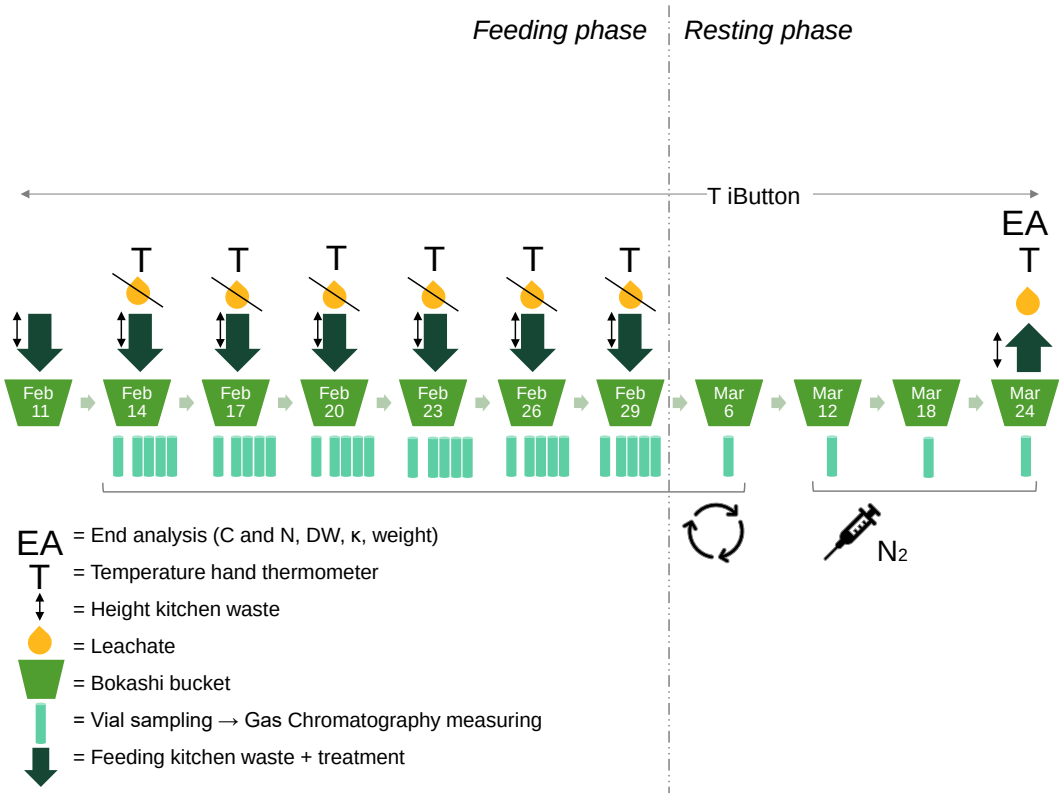

Figure 2: Experimental schedule with feeding and vial sampling.

2.2.2. Feeding

Equal amount of kitchen waste (1401 g) was added to all buckets every third day in the feeding phase. The kitchen waste contained cleaned shredded potatoes and dried cat food with a weight ratio of 2:1 (Table 2). The dry weight of the analysed potatoes and the cat food was 24.7 % and 93.3 %, respectively. The potatoes differed in quality (mouldy) and varieties over the experiment. At the fifth feeding 400 g peel was used in the mixture. The kitchen waste C/N ratio was calculated from the analysed ingredients to 12.26.

Table 2: C and N properties of ingredients in substrate showing total Nitrogen (tot-N), total Carbon (tot-C), dry substance (DS) and Carbon to Nitrogen ratio (C/N).

	tot-N (%)	tot-C (%)	DS	C/N
Potatoes	0.92	41.27	0.25	44.66
Cat food	5.24	48.45	0.93	9.24
Kitchen waste	3.75	45.97	0.48	12.26
EM Bokashi bran	5.68	87.97	0.33	15.49

The buckets were opened for approximately 5 minutes during feeding to be able to measure temperature, and height of food waste before and after adding additional food waste. EM Bokashi and autoclaved EM bokashi was also added to respective

treatments each feeding according to the manual (*Bruksanvisning EM Bokashihinkar* 2018) of Bokashiworld in Sweden AB. That equal 9 g for EM Bokashi and 3 g for Autoclaved each time.

The kitchen waste was prepared the day before the feeding. The potatoes were shredded with a food processor (*Philip Storemaster electronic)* using the coarsest size option. It was mixed with the cat food and stored in plastic bags overnight before feeding.

2.2.3. Sampling

Feeding phase

During the feeding phase (11-29 Feb), gas samples were taken every third day (14th, 17th, 20th, 23rd, 26th, and 29th of February). All samplings were performed in the morning and feeding in the afternoon the same day. This was to be able to measure before opening the lid, and after according to a time manual. Andersen *et al.* (2010) also measured before adding waste in their study of compost.

The tubes were connected to a pump and to a vial (Figure 3). The measurements were taken over 30 s to ensure mixing. That gave an air change in the pump system and vial of 7 times to give accurate results. Between each bucket the pump was aeriated in the room for at least 30 s.

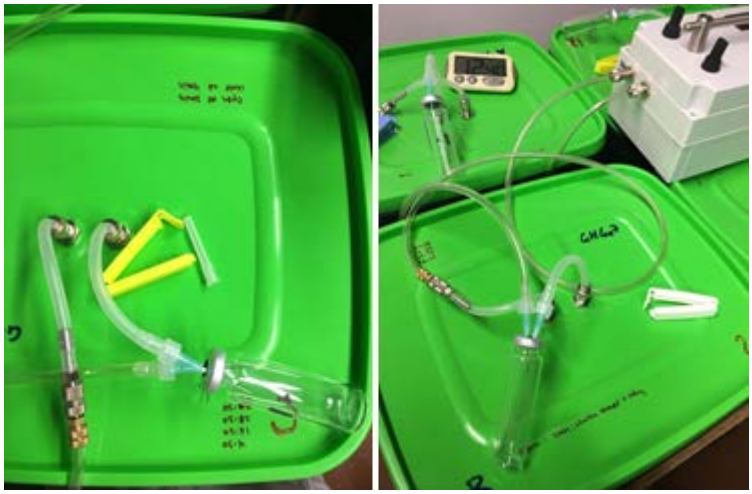

Figure 3: Sampling of GHGs during the feeding phase.

One sample was taken from each bucket before opening the lid.

Thereafter the buckets were divided in to two timeseries measuring emissions from 6 buckets at a time. Four samples were taken from each bucket in a timeseries with 12 minutes between measurements in the same bucket (see Appendix I for schedule). Each bucket was individually aeriated for 2 minutes and closed for 30 s before their first sampling, as a part of the timeseries schedule. The four timeseries samplings were used as a flux estimation, using linear regression analysis. The last samplings (Feb-29) were done with a

shorter time lap of 3 min, one bucket at a time, to still fit the calibration curve of the gas chromatography (GC).

Resting phase

During the resting phase gas samples were taken every sixth day (6th, 12th, 18th and 24th of March). The first time it was done in the same way as during the sampling prior to opening the lid during the feeding phase, i.e., with the pump sampling for 30 s in each bucket. No timeseries nor opening of the lid was performed. For the following samplings (12th, 18th and 24th of March) a new method was developed to prevent additional air in the system that could inhibit methanogenesis (Figure 4). The pump was replaced by a 5 ml syringe filled with N_2-gas to minimize the amount added.

Figure 4: Sampling during resting phase using a syringe filled with 1 atm overpressure of N_2 gas.

First, the plastic fittings on the gas port tube on the lids were switched to 3-way stopcocks. The syringe was connected to an additional 3-way stopcock rinsed 10 times and then filled with N_2 with 1 bar overpressure. Additional N gas was emptied to 5 ml and was then connected to the vial and 3-way stopcock on the lid. The syringe was emptied and filled 20 times in the bucket to create a mixture in the bucket, and then emptied in the vial for further GC analysis.

2.2.4. GHG measuring and analysis

CH_4, N_2O and CO_2 were analysed with gas chromatography (GC) with a PerkinElmer Headspace Sampler TurboMatrix 110. Detectors used were electron capture (ECD) for N_2O and flame ionisation (FID) for carbon containing gases. Results were given in mole ppm. CO_2 concentration was additionally measured with a handheld CO_2 meter from Vaisala, connected directly to the buckets.

During the feeding phase the emissions were estimated from sampling after three days, compared to background levels in the room. Additionally, estimations of emissions during feeding were added to the levels. The formula used for estimating emissions when opening the lids, followed (Andersen et al. 2010) and are as follows:

$$E_{gas} = \frac{dC_{gas}}{dt} \times V_{headspace}$$

Where E is emissions in µg gas/min (g gas/min for CO_2), C is concentration (µg gas $L_{headspace}^{-1}$), t is time (min) and V volume (L).

The emissions over the whole fermentation the following formula (Andersen et al. 2010) was used:

$$EF_{gas} = \sum \int \ (E_{gas}\, dt)/m_{input}$$

EF is emission factor (g/kg ww), t is time (min), and m is mass (kg). dC_{gas}/dt is the linear regression calculated from the time series in ppm/min. EF takes both the mass of added ww and the headspace volume into consideration to make all the emissions comparable.

The integral for the resting phase is the same as calculated values after two weeks (minimum time recommended for Bokashi). Calculations were based on exponential curves (the best fit) of the resting phase measurements. The background levels of the ambient air were subtracted from the measured values (N_2O=0.30, CH_4=1.88, CO_2=648 ppm).

Conversion of measured mole ppm gas to g gas/L was calculated with the help of the Ideal Gas Law:

$$C_{m/V} = \frac{C_{mole\ ppm} MP}{RT} /1000$$

Where $C_{m/V}$ is concentration (g gas $L_{headspace}^{-1}$), $C_{mole\ ppm}$ is concentration (mol mol^{-1}), M is Molar mass of the GHG (N_2O=44.013 CH_4=16.04 CO_2=44.01 g mol^{-1}), P is standard pressure (101325 Pa), R is the universal gas constant (8.31451 m^3 Pa K^{-1} mol^{-1}), T is temperature (K), 1000 was to convert from m^3 to L.

The headspace volume was calculated by subtracting the volume food waste from the total volume. Food waste volume was estimated by measuring the height of added food waste in four points approximately 3 cm from the edges near the corners. To compensate for the tilting edges of the bucket, the following formula was used for volume estimations:

$$V_{input} = \left(D_{input} \times \frac{\Delta L/\Delta D}{2} + L_{lower}\right) \times (D_{input} \times \frac{\Delta W/\Delta D}{2} + W_{lower}) \times D_{input}$$

Where V_{input} in the volume of the food waste, D is depth, L is length, and W is width. Lower refers to the lower part of the bucket.

2.2.5. Temperature, pH, moisture, C/N ratio

Temperature

Temperature was measured throughout the experiment in two ways, both with iButton set for measurements every 30 min and with a hand thermometer before feeding with additional substrate. The iButton records the temperature in the headspace of the bucket and the hand thermometer in the substrate.

Leachate analysis and pH

When leachate was produced, pH and electrical conductivity were measured in the leachate, using a multimeter (WTW Multi 1970i).

Water content

The water content was measured in the separate ingredients of the substrate prior the start of the experiment with weighing before and after freeze drying. This was repeated for the homogenised end product after the experiment. Moisture was also measured in the upper six cm of the substrate in the buckets with a HH2 Moisture Meter connected to a Theta Probe ML2x.

C and N

C and N content were analysed in the substrate ingredients and in homogenised end product. One variety of potatoes was used for this analysis, while data for King

Edward potatoes were taken from The Swedish National Food Agency report *Potatis - analys av näringsämnen*. C and N analysis was also made for the leachate.

C and N balance was calculated both based on mass and on measured emissions. The mass C and N at the start and end of the experiment was calculated from the tot C or N (%) in the analysis:

$$C\ or\ N_g\ = m_{ww_g} \times DS \times tot\ C\ or\ N(\%)$$

Where m is the mass of the wet waste in g, DS the dry substance and C or N (%) the percentage of the dried mass.

The measured losses are the sum of C or N emitted as GHGs and leachate. The C and N in the leachate was calculated from the analysis Total C and N. N_2O, CH_4 and CO_2 was converted to N_2O-N, CH_4-C and CO_2-C respectively. The molar weight (M) of 2×N (28.01 g $mol^{-1)}$ and C (12.01 g mol^{-1}) was divided by the M of the molecule N_2O=44.013 CH_4=16.04 CO_2=44.01 g mol^{-1}, respectively.

2.2.6. Total emissions

For calculations of total emissions CO_2-equvalents were calculated both with the IPCC lower and higher GWP values, with the following formula:

$$m_{CO_2} = m_{gas} \times GWP_{gas}$$

Where m is the total mass gas during the experiment, and GWP is global warming potential (CO_2-equivalents).

2.3. Statistical analysis

Linear regression analysis was performed for the timeseries (1-4) in Microsoft Excel. Single ANOVA with Tukey's HSD test was performed in RStudio, for comparisons of GHG emission between the treatments. The assumption of homogeneity of variance was tested in RStudio with Bartlett's test. When the requirement was not met, a Welch's ANOVA test was performed instead. Significance levels were set to 0.05, for the post hoc test with the Benjamini-Hochberg adjustment.

3. Results

3.1. Literature review

3.1.1. Bokashi.se report

One linked reference comparing composts and EM Bokashi can be found linked from bokashi.se (*Bokashiword in Sweden AB, 2020*). The study is made for EM Agriton BV by Feed Innovation Service BV, but no scientifically published article could be found of this project. The experiment compares compost with Bokashi treatment, for roadside mowing windrows. In the Bokashi treatment clay mineral and seashell lime was also added. Their conclusion was that the fermentation part of Bokashi produces considerably less GHG emissions than composting. The conclusion was based on measuring of soil loss and the assumption that the proportion of CH_4 and CO_2 is the same in compost as in EM Bokashi. GHGs were not measured.

3.1.2. Peer reviewed reports

None of the studies found in this review have examined EM Bokashi explicitly as a method of reducing GHG emissions. However, there are examples when Bokashi have been tested for biogas production (Hanafiah 2017). There are also example of CO_2-measurements used as an indicator for basal soil respiration in field studies of Bokashi (Mayer, 2010; Shin, 2017). One review report compared different treatment of organic waste, where GHG emission was one variable (Bortolotti et al. 2018).

Measurements of CH_4 were only found in studies concerning biogas production. Hanafiah (2017) results showed that no CH_4 was produced. In that study goat and chicken manure was used. The explanation given by Hanafiah (2017) is that their Bokashi treatment produces large amount of CO_2, ammonia (NH_3) and hydrogen sulphide (H_2S), which would limit the methanogens growth. The pH was also measured during their experiment showing acidic conditions for the Bokashi treatment (pH 3.55-4.42).

One review study reason that there is a theoretical risk of N_2O production if the substrate has a high ammonium content, and suggest that easily accessible C should be applied to prevent N loss (Quiroz & Céspedes 2019). In that study the term bokashi was used in a broader sense with variation of selected microorganisms that

included *Streptococcus lactis, Candida utilis, Streptomyces albus, Streptomyces griseus, Aspergillus oryzae* and *Mucor hiemalis*. Both aerobic and anaerobic conditions were taken into consideration also.

When it comes to CO_2 one Swiss field study, examining the effect of EM on yield, showed that there was no significant difference in emissions from autoclaved Bokashi compared to living Bokashi. Neither were EM spray treatments compared to the control (water) significant (Mayer et al. 2010). Respiration seemed to be more connected to the organic input than EM over the experiment that was performed over four years in a temperate climate. A Belgian study on sandy soils showed that the respiration after one week was significantly higher for Bokashi and autoclaved Bokashi compared to a control sample (Shin et al. 2017). Measurements after eight weeks showed no significant difference though. Quiroz & Céspedes (2019) bring up respiration indirect by reviewing mineralisation rates from EM treated composts. One of the studies saw an increase in mineralisation with a decrease in C and C/N ratio (Jusoh et al. 2013), while no effect was found in another study (Formowitz et al. 2007).

Bortolotti *et al.* (2018) claim that lactic acid fermentations in general and Bokashi explicitly releases relatively high GHG emissions compared to other groups of decentralised treatment of organic waste such as composting, anaerobic digestion, dehydration, mulching and vermicomposting. In the study's appendix it is stated that for some of the treatments results might rest on grey literature, but for GHG emissions from Bokashi it is based on scientific literature.

3.2. Laboratory study

3.2.1. GHG emissions

N_2O

During the feeding phase, the first two measurements showed low emissions, followed by higher emissions over the rest of the phase. There was a significant difference in emissions the 29th of February (p=0.02), with lowest values for the EM treatment. The Tukey's HSD test showed a significant difference between the Control and EM treatment. The total emissions over the feeding phase did not

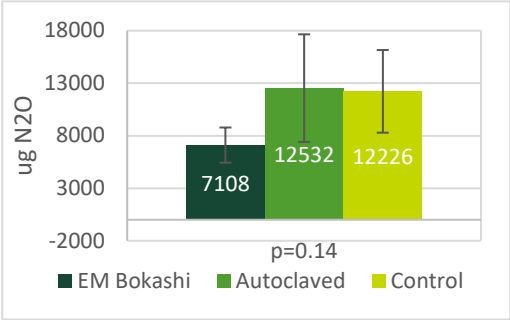

Figure 5: Mean ($\bar{x}$) and standard deviation for the total N_2O emitted over the feeding phase, based on the sum of accumulated levels after 3 days and 2 min feeding with open lid. The p-value is from the anova analysis comparing the different treatments.

differ significantly, but a trend for lower emissions in the EM treatment was observed (Figure 5).

For the first three timeseries measured, the linear regression analysis generally gave negative fluxes with low r^2-values. The 4th to 6th measurements showed positive flows with r^2-values over 0.9 except for three series the 4th measuring (Autoclaved 2(0.79) and 4(0.07), Control 2(0.49)). All fluxes with r^2-values below 0.6 were set to 0. The mean of total emissions when feeding for 2 minutes/time and maximum emission (E_{max}) are shown in Table 3. The total amount released when feeding is negligible compared to the accumulated values after three days.

Table 3: N_2O emissions from time series regression of 2 minutes, giving the total mean for all feedings, standard deviation and the highest emission measured.

	$\Sigma(E_{N_2O} \times dt)$		E_{max}
	$\bar{x}$ (µg)	SD	(µg/min)
EM Bokashi	42.18	4.78	12.1
Autoclaved	58.91	22.29	16.3
Control	61.27	7.56	19.3

The Bartlett's test could not confirm homogeneity in variance during the resting phase, so a welch test was performed. It showed significant difference for all measuring times (p=0.046, 0.011, 0.001 and 0.002). The post hoc games Howell test only showed a significant difference the 6th of March between the EM and Control treatment. The samples distribution and mean values are shown in mole-ppm (Figure 6).

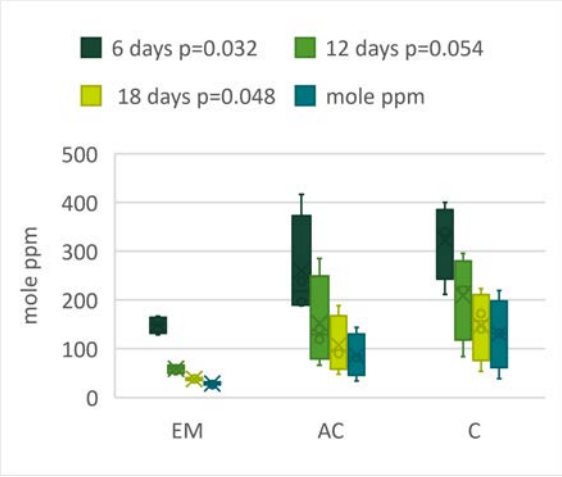

Figure 6: Sample distribution and mean(x) for N_2O emissions during the resting phase after 6, 12, 18 and 24 days. P-values are from the welch test. EM=EM Bokashi, AC=Autoclaved, C=Control treatment. Different colours denote days.

The highest N_2O levels in the resting phase was measured after six days, followed by a decrease in concentrations with time. The best match was an exponential decrease. This means that after two weeks EM Bokashi released between 52-67 ppm N_2O, the Autoclaved released between 74 and 253 ppm, and the Control 85-270 ppm (Figure 7). This is equivalent to 333-375 ($\bar{x}$=342, SD=23), 436-1525 ($\bar{x}$ =868, SD=466) and 476-1660 µg N_2O ($\bar{x}$ =1152, SD=493).

Accumulated N_2O emissions over the whole fermentation phase is shown in Figure 8. EM have the lowest mean (7450 µg, SD=1686) measured, followed by Control (13378 µg, SD=4151) and Autoclaved (13400 µg, SD=5377). The differences were not significant according to the Welch test (p=0.077). The adjusted p-values were 0.17(EMAC), 0.17(EMC), and 1.00(ACC).

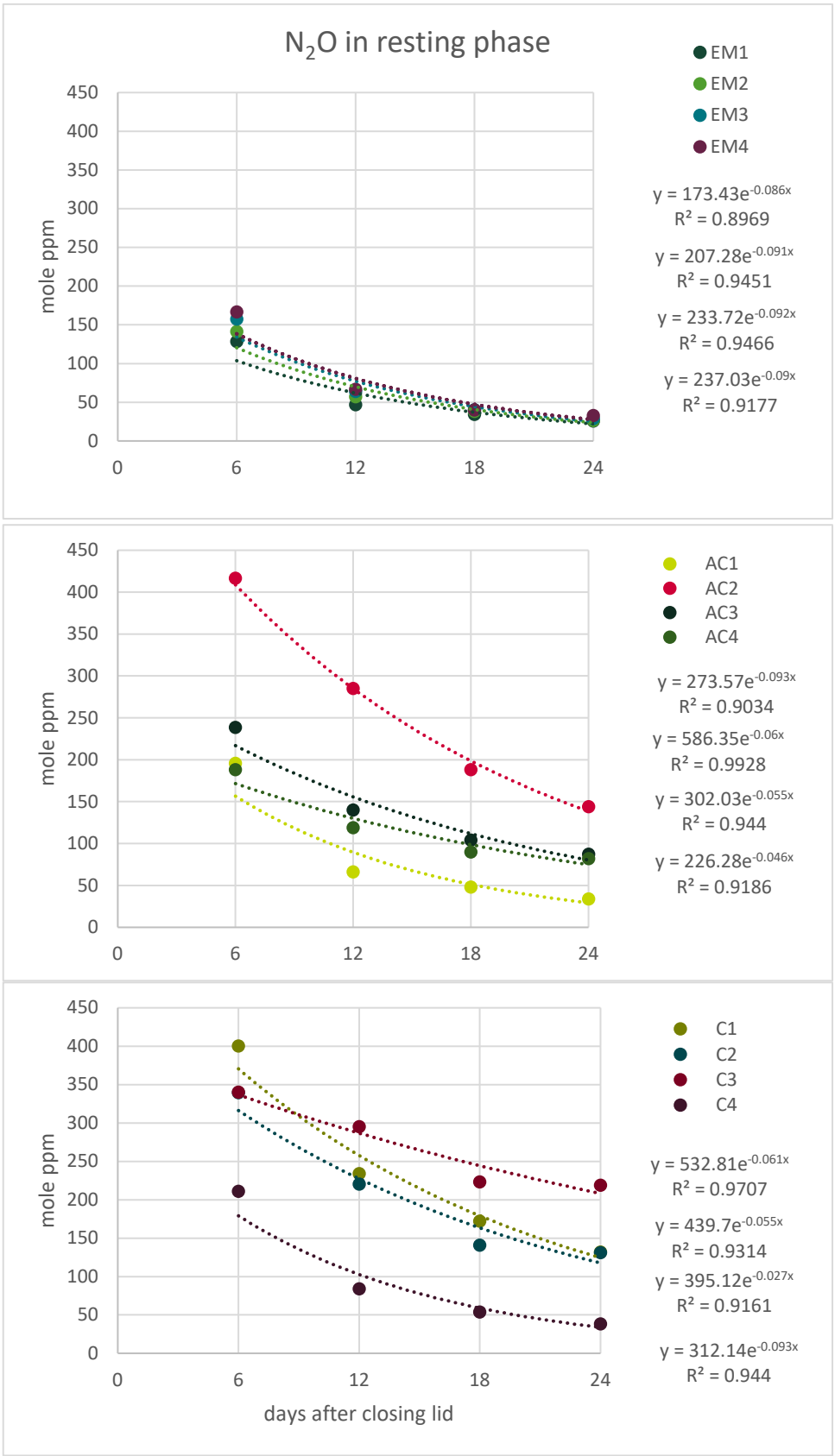

Figure 7: N_2O emissions (mole ppm) during the resting phase for EM Bokashi (EM1-EM4) Autoclaved (AC1-AC4) and Control treatment (C1-C4), with respective equations for exponential decrease over time.

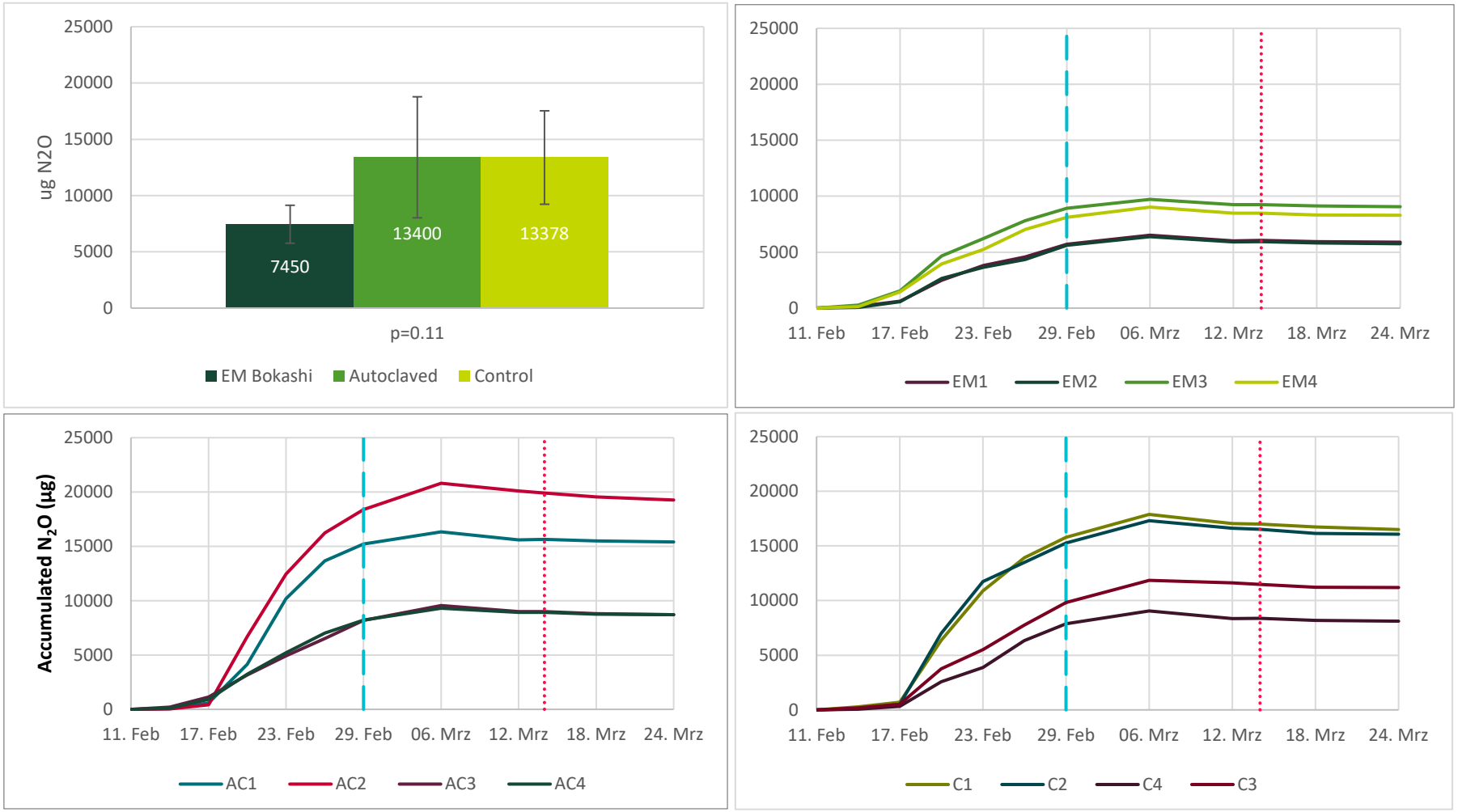

Figure 8: Accumulated N_2O emissions over the whole fermentation phase. EM =EM Bokashi, AC=Autoclaved, and C=Control (kitchen waste without treatment). Dashed line= border between feeding and resting phase. Dotted line= 2 weeks of resting. Bar chart showing mean and SD for the total emissions until the 14th of March. p-value is from the ANOVA test.

CH_4

During the feeding phase, measured ppm kept around the background levels for the first 2-3 samplings. Emissions were thereafter detected for the rest of the feeding phase. No significant difference in emissions were found in the feeding phase, neither for sampling separately nor for the total accumulated emissions (p-values=0.75, 0.84, 0.94, 0.78, 0.17, 0.45, and 0.34). The mean of the total emissions were highest for the EM treatment, followed by Autoclaved, and lowest for the Control treatment (Figure 9).

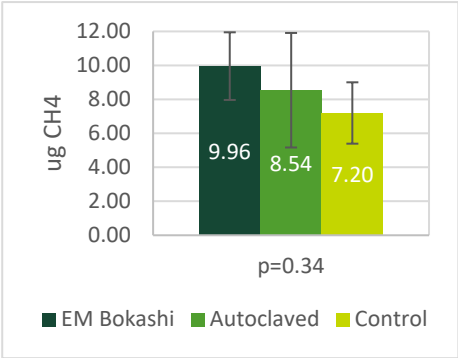

Figure 9: Mean ($\bar{x}$) and SD for the total CH_4 emitted over the feeding phase, based on the sum of the accumulated levels after 3 days and 2 min open lid for each feeding. The p-value is from the ANOVA analysis comparing the treatments.

The regression curves confirmed the trends with low r^2-values for the first three samplings, followed by higher for the rest of the feeding phase. Highest ug/min was found in EM treatment, followed by the Control, but EM had the lowest mean of the total emissions from the regressions (See Table 4).

Table 4: CH_4 emissions during feeding based on time series regression, giving the total mean fort all feedings, standard deviation and the highest emission measured.

	$\Sigma(E_{CH_4} \times dt)$		E_{max}
	$\bar{x}$	SD	(µg/min)
EM Bokashi	0.03	0.035	0.018
Autoclaved	0.05	0.016	0.015
Control	0.05	0.021	0.018

In the resting phase (Figure 10) an increase was detected from the beginning to the third measurement. For the last measurement the CH_4 levels were low again for all buckets. Two estimations of emissions after two weeks were made, one based on an exponential fit of the first three measurements, and one without the third measurement.

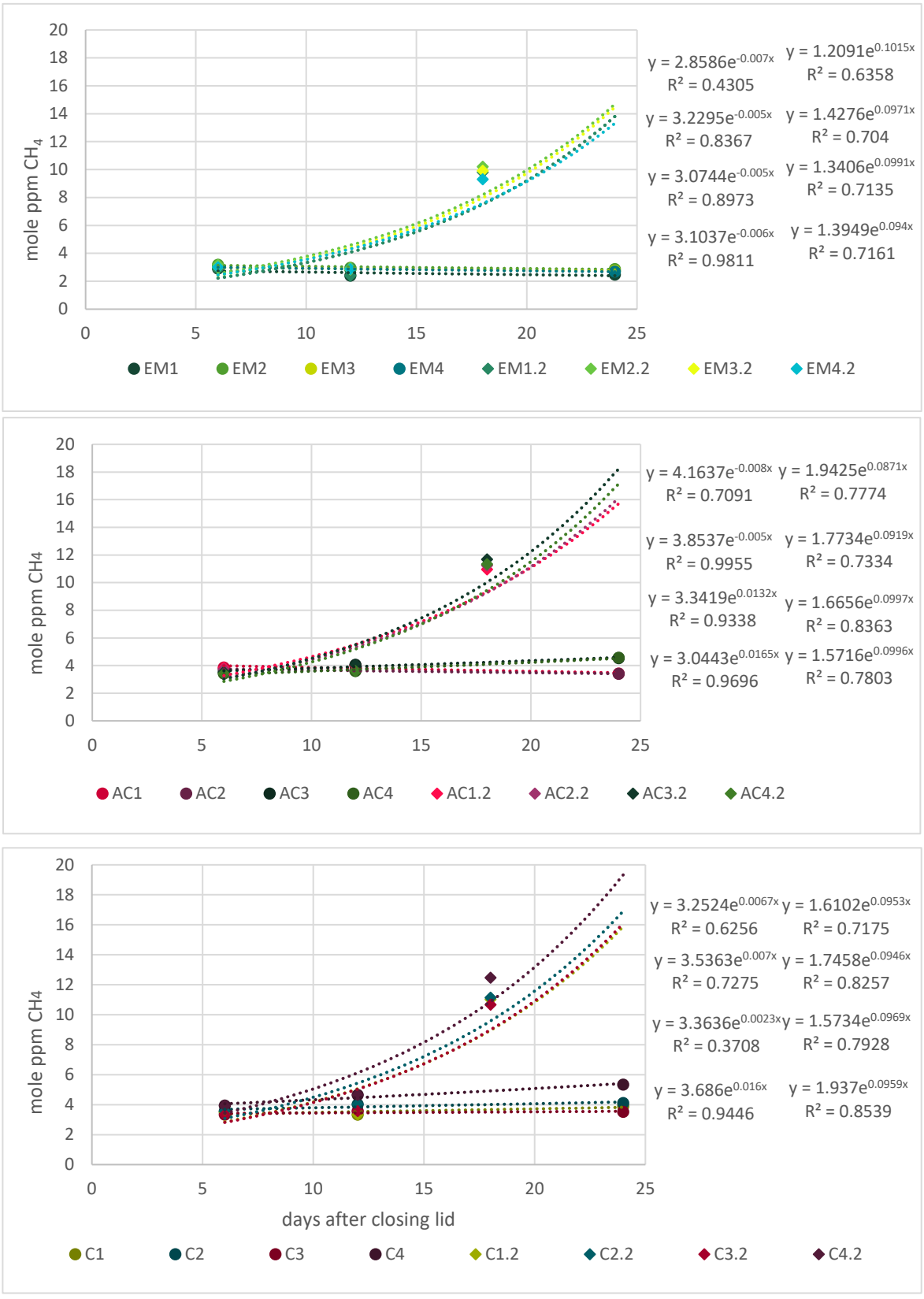

Figure 10: CH_4 levels in headspace during the resting phase for EM Bokashi, Autoclaved, and Control treatment. Trendlines with equations are based w/o 3rd or 4th sampling.

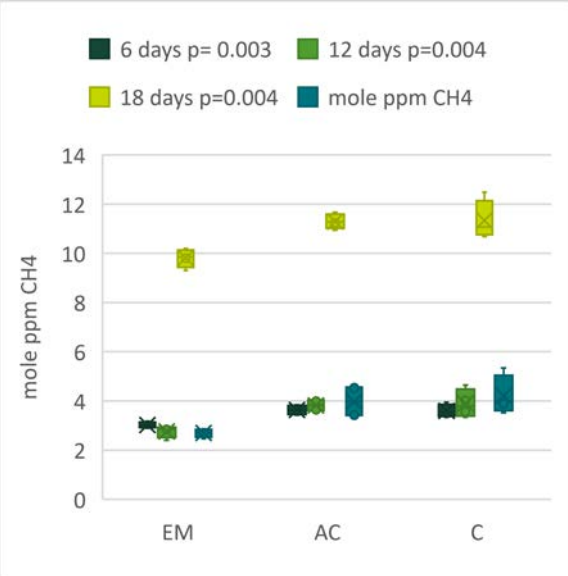

Figure 11: Sample mean(x) and distribution for CH_4 emissions during resting phase for the different treatments EM Bokashi (EM), Autoclaved (AC) and Control (C) after 6, 12 and 18 days respectively. The p-values are from ANOVA test.

The accumulated CH_4 levels for EM, Autoclaved and Control treatment after two weeks were 5.0-5.6, 6.3-6.7 and 6.1-7.4 ppm mole (Figure 11), or recalculated with subtracted background values 6.6-7.5, 9.5-10.3 and 8.3-11-3 µg CH_4.

The ANOVA showed a significant difference between the treatments, for all measuring times separately (Figure 11). The Tukey HSD t-test showed a significant difference both when comparing EM to the Autoclaved and Control treatment.

The accumulated measured emissions over the whole feeding phase can be seen in Figure 12.

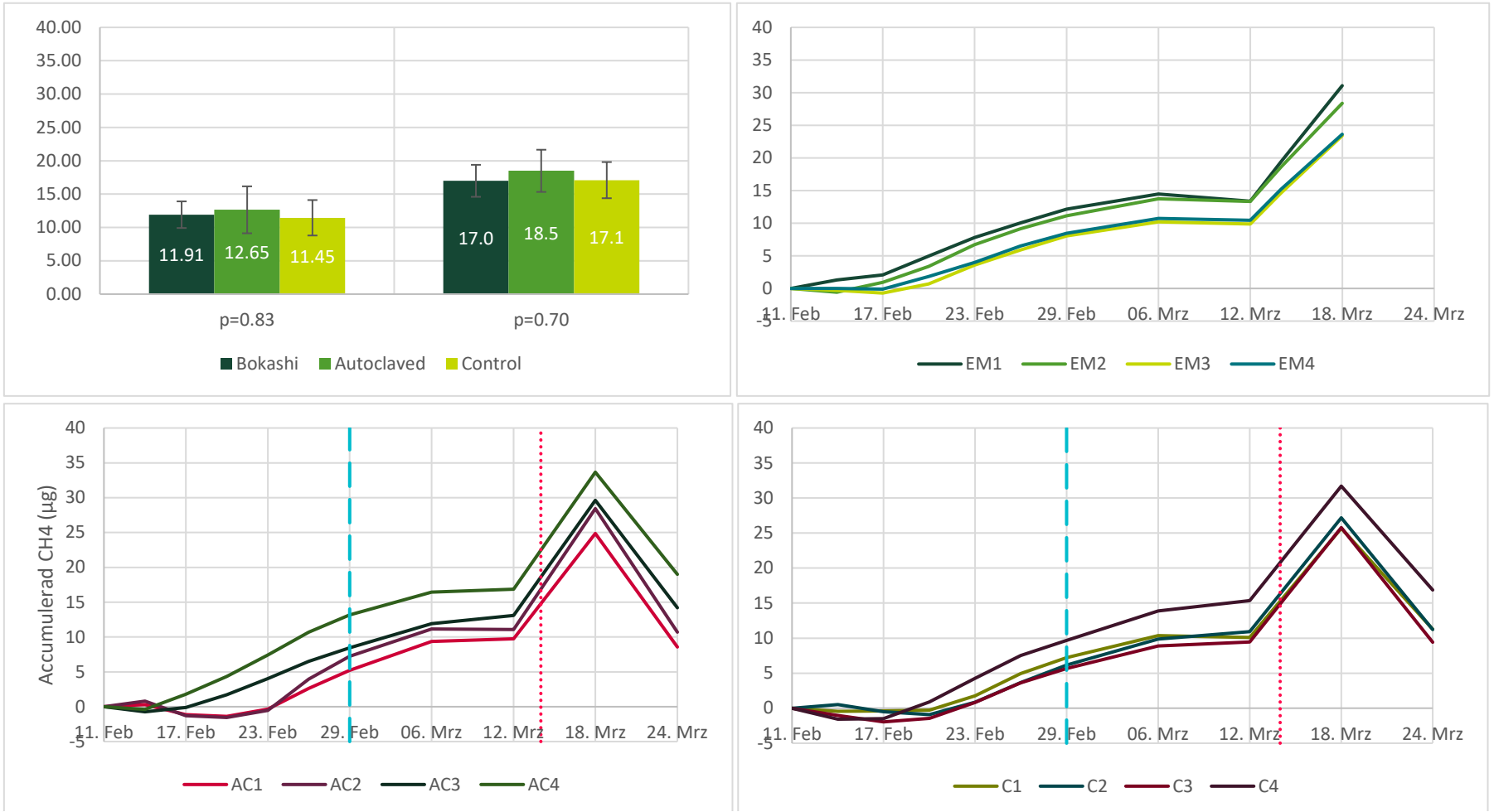

Figure 12: Accumulated CH_4 emissions over the whole fermentation phase. EM =EM Bokashi, AC=Autoclaved, and C=Control (kitchen waste without treatment). Dashed line= border between feeding and resting phase. Dotted line= 2 weeks of resting. Bar chart showing mean and SD for the total emissions until the 14th of March, w/o 3rd and 4th measurements respectively. p-value is from ANOVA test.

CO_2

The direct measuring of CO_2 showed high concentrations with results out of scale for the CO_2 meter (approximately 45000 ppm). The GC also showed high emissions in all buckets with much higher values than the highest standard (>10000 mole ppm) for the instrument. The uncertainty was constantly high and varied between 29 and 66 rel% (from first analysis: 33; 38; 29; 43; 45; 37; 66; 66; 66; 25rel%).

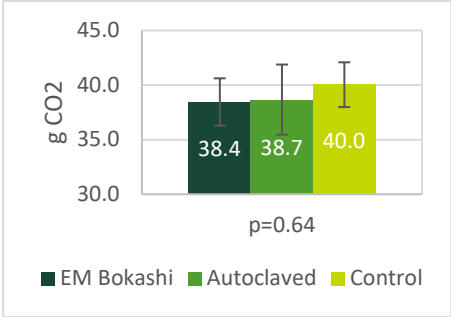

Figure 13: Mean ($\bar{x}$) and SD for the total CO_2 emitted over the feeding phase, based on the sum of the accumulated levels after 3 days and 2 min open lid for each feeding. The p-value is from the ANOVA analysis comparing the treatments.

None of the separately measured emissions, nor the cumulative CO_2 emissions over the feeding phase differed significantly between the treatments (p =0.79, 0.87, 0.81, 0.67, 0.75, 0.72 and total 0.64) (Figure 13). The regression curves gave highest emissions from the EM treatment, followed by the Control and lowest for the Autoclaved (Table 5).

Table 5: CO_2 emissions during feeding based on time series regression, giving the total mean fort all feedings, standard deviation and the highest emission measured.

	$\Sigma(E_{CO_2} \times dt)$		
	$\bar{x}$ (g)	SD	E_{max} (g/min)
EM Bokashi	0.23	0.02	0.07
Autoclaved	0.15	0.06	0.06
Control	0.17	0.02	0.05

In the resting phase, the highest CO_2 emissions were measured from the EM treatment (Figure 14 and 15). The ANOVA showed a significant difference from all measurements separately and the Tukey's HSD test confirmed this for all comparisons between the EM and the Autoclaved treatment. When comparing EM to the Control treatment the test showed significant difference for 12 and 18 days of resting.

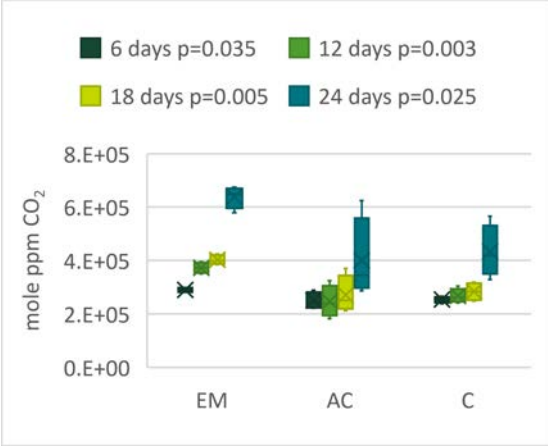

Figure 14: Sample mean(x) and distribution for CH_4 emissions during resting phase for the different treatments EM Bokashi (EM), Autoclaved (AC) and Control (C) after 6, 12 and 18 days respectively. The p-values are from ANOVA test.

The accumulated measured CO_2 emissions can be seen in Figure 16.

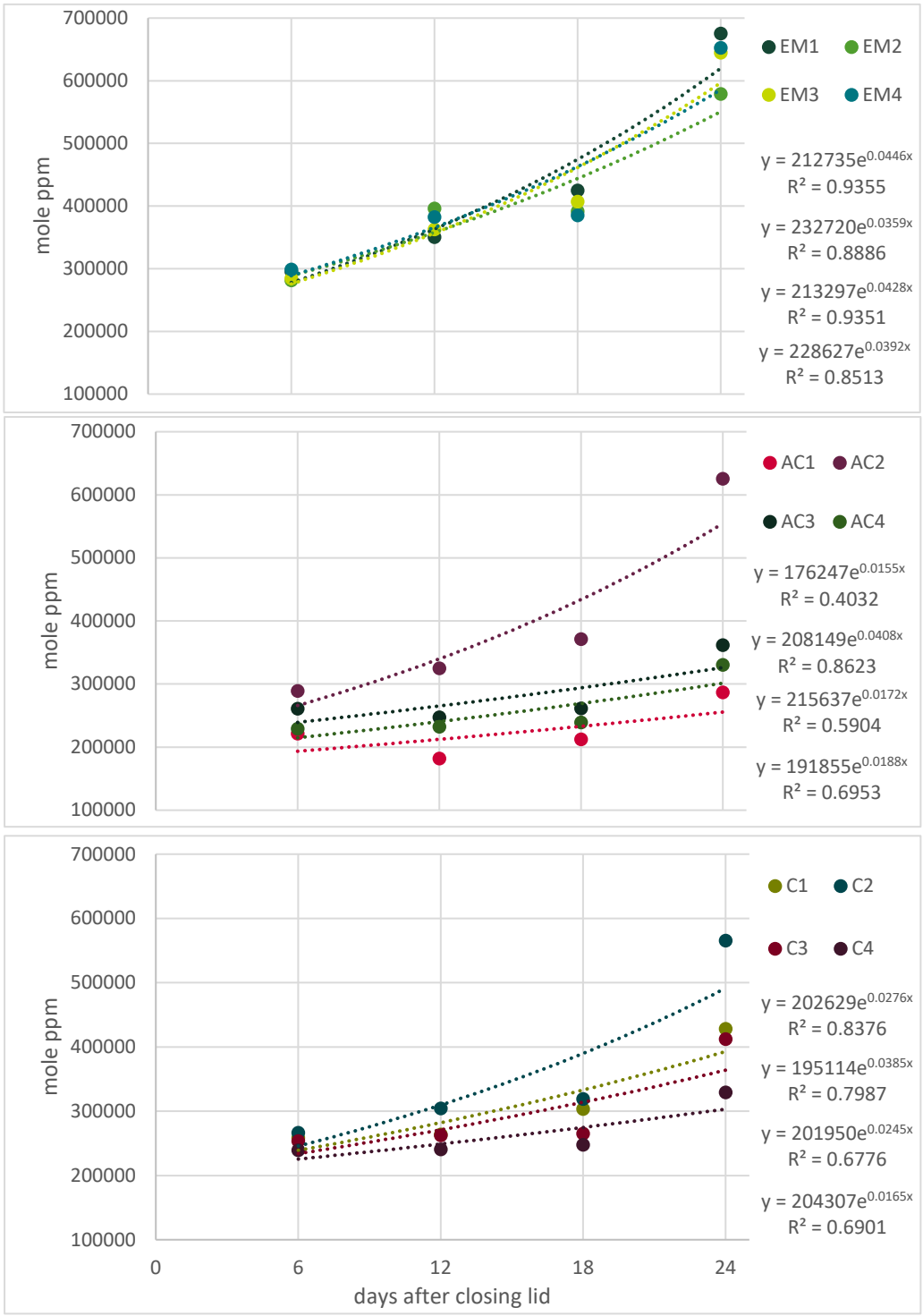

Figure 14: CO_2 levels in headspace during the resting phase for Bokashi (B1-B4), Autoclaved, (AC1-AC4)and Control treatment(C1-C4), with respective equations for exponential decrease over time.

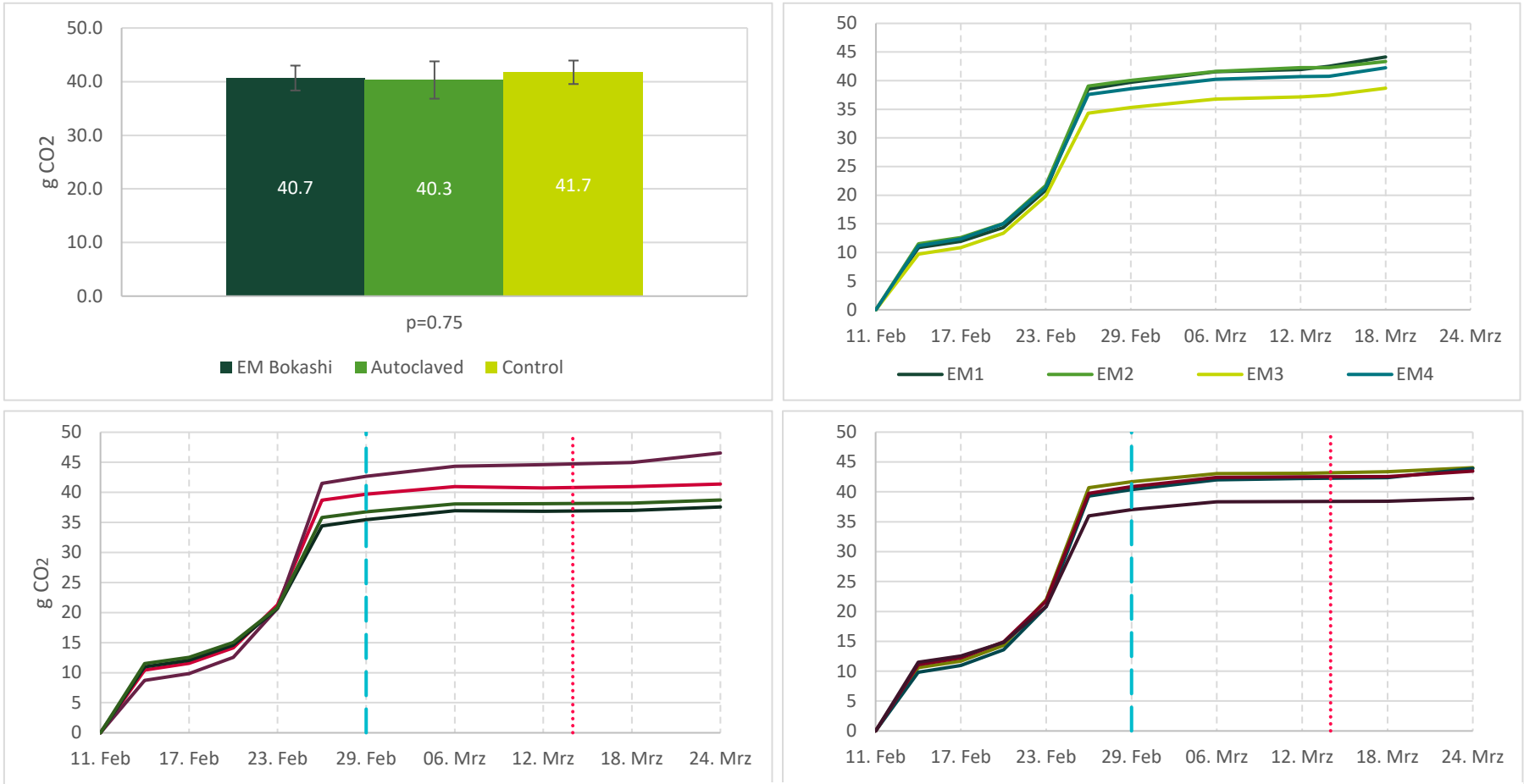

Figure 15: Accumulated CH_4 emissions over the whole fermentation phase. EM =EM Bokashi, AC=Autoclaved EM, and C=Control (kitchen waste without treatment). Dashed line= border between feeding and resting phase. Dotted line= 2 weeks of resting. Bar chart showing mean and SD for the total emissions until the 14th of March, the p-value is from the ANOVA test.

3.2.2. Emission factors

The Emission factors (EF) over the fermentation phase were 1.01-1.40, 0.93-1.77 and 0.94-1.56 µg CH_4-C/kg wet waste for EM Bokashi, Autoclaved and Control treatment respectively. The N_2O for the same treatments were 0.62-0.94, 0.91-2.0 and 0.86-1.74 mg/kg wet waste (Table 6). CO_2 emissions were 4.13-4.28 (EM Bokashi), 3.77-4.57 (Autoclaved) and 3.93-4.42 g/kg wet waste (Control). This gives a total global warming EF of 4.06-4.46, 4.04-5.17 and 4.18-4.94 CO_2-eq/kg ww.

Table 6: Emission factors (EF) for all treatments over the fermentation phase, together with calculations for tot global warming EF, based on IPCCs GWP 34 for CH_4 and 298 for N_2O.

	CH_4 gas	N_2O gas	CO_2 gas	Total EF (CO_2-eq/kg ww)	
	g/kg ww	g/kg ww	g/kg ww	Excl. CO_2 gas	Incl. CO_2 gas
EM Bokashi 1	1.40E-06	6.15E-04	4.28	0.18	4.46
EM Bokashi 2	1.36E-06	6.03E-04	4.28	0.18	4.46
EM Bokashi 3	1.01E-06	9.39E-04	3.78	0.28	4.06
EM Bokashi 4	1.06E-06	8.60E-04	4.13	0.26	4.39
Autoclaved 1	9.32E-07	1.59E-03	4.17	0.48	4.64
Autoclaved 2	1.12E-06	2.03E-03	4.57	0.60	5.17
Autoclaved 3	1.33E-06	9.20E-04	3.77	0.27	4.04
Autoclaved 4	1.77E-06	9.10E-04	3.89	0.27	4.16
Control 1	1.08E-06	1.74E-03	4.42	0.52	4.94
Control 2	1.09E-06	1.69E-03	4.33	0.50	4.83
Control 3	9.44E-07	1.17E-03	4.35	0.35	4.70
Control 4	1.56E-06	8.57E-04	3.93	0.26	4.18

3.2.3. Temperature, pH, moisture, C/N ratio, visual observations

Temperature

Two out of 4 iButtons worked, one for the Autoclaved, and one for the Control treatment. The temperature in the Autoclaved treatment and in the bucket without Bokashi bran fluctuated between 18 and 21°C (Figure 16).

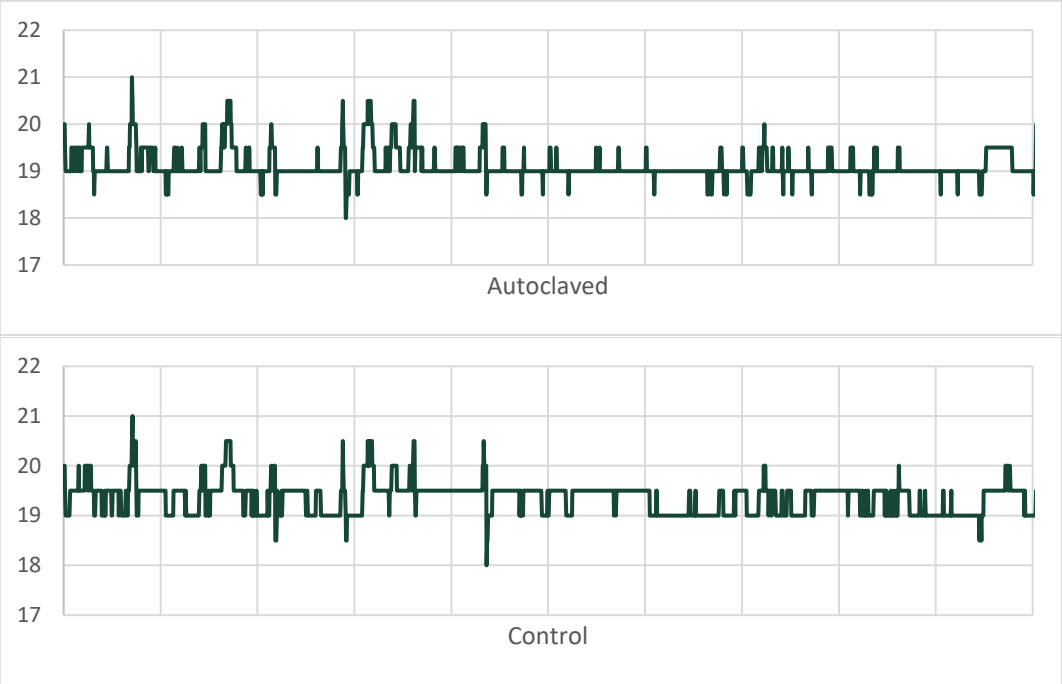

Figure 16: Headspace temperature (°C) measured with iButton for the Autoclaved and Control treatment between Feb-11 and Mar-24.

The measurements with a hand thermometer showed no significant difference between the treatments. The highest temperature was measured the 14th of February in the beginning of the experiment (between 21.2 and 22.1°C). It decreased to 20.7-20.9°C to the 23rd, having a small peak at the 26th and going down to the same levels on the 29th of February (Figure 17). The measurements after the ending of the experiment (24th of Mars) showed temperatures between 20.5-20.8°C with the mean of 20.7°C regardless of treatment. In general, the temperatures were higher in the substrate than in the ambient air.

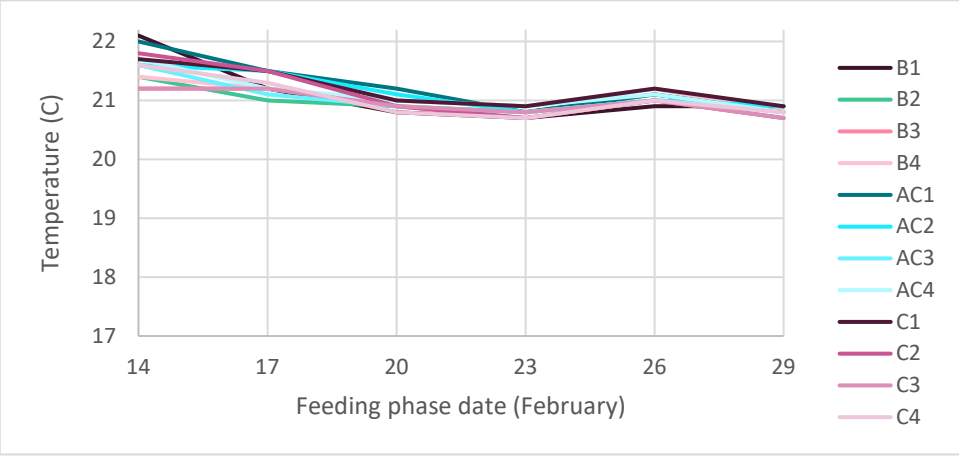

Figure 17: Temperatures (°C) measured with hand thermometer in substrate over the feeding phase, showing similar temperatures for all treatments.

Leachate analysis and pH

During the feeding phase, no leachate had developed from any of the treatments. After the resting phase all treatments had developed leachate. The EM developed 145-200 ml, the Autoclaved 170-260 ml and the Control 160-300 ml. The pH was below 5, 4.37-5.94 and 4.64-5.7 in the EM, the Autoclaved and the Control leachate, respectively (Table 7). There was however no significant difference in pH. The EM leachate differed in colour compared to the others (Figure 18) and left long slimy threads from the tap. The EM was orange yellow and the others brown. There was no significant difference in pH, nor in electrical conductivity.

Figure 18: Leachate at the end of the experiment. Showing from the left: EM Bokashi (1-4), Autoclaved (5-8), Control (9-12). Colour difference can be seen between EM treatment and the rest.

Table 7: Leachate properties at the end of the experiment showing mass (g), volume (ml), pH and electrical conductivity (κ).

	EM Bokashi				Autoclaved				Control			
m(g)	221	218	160	135	181	239	179	194	174	257	191	266
V(ml)	200	200	150	145	175	260	170	185	160	300	185	300
pH	4.57	4.72	4.88	4.81	5.21	5.94	4.67	4.37	5.7	5.28	4.64	4.72
κ(mS/cm)	30.6	30	32.7	31.4	32	32.3	30.4	29.7	33.3	32.5	30.5	29.7

Analysis for C and N are shown in Table 8. One separate sample was taken from Autoclaved 1, since colonies of white mould was observed on the waste for that treatment. The sample from Control 1 was excluded from the test since autoclaved bokashi was added to that bucket on one occasion. NO_2 and NO_3 concentrations were much higher in the EM leachate compared to the other treatments, but less leachate was produced. Total N was lower for the Control compared to the Autoclaved and EM treatments.

Table 8: C and N analysis with Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), Total Nitrogen Bound (TNb), Ammonium (NH_4) and Nitrite (NO_2) +Nitrate (NO_3).

	TOC g/l	DOC g/l	Tot-N_TNb g/l	NH_4_N g/l	NO_2+NO_3_N mg/l
EM Bokashi	57.1	53.3	8.78	1.64	2.97
Autoclaved	56.5	53.6	8.51	1.61	1.93
Control	50.8	45.2	7.75	1.48	1.86
Autoclaved w. mould	55.0	51.5	8.71	1.91	1.66

Water content and moisture

The moisture measured in the top 6 cm was around 50 vol%, and the water content of the homogenised product was just above 50 mass%, for all treatments (Table 9).

Table 9: The moisture of the product. Vol% is measured in the top 6 cm and the mass% from the homogenised product.

Moisture	EM Bokashi				Autoclaved				Control			
vol%	48.6	48.3	47.9	50.0	50.1	47.5	47.4	46.1	48.9	48.4	48.4	47.8
mass%	56.1	53.9	54.6	55.2	53.0	53.8	54.4	53.7	54.5	56.0	54.1	53.9

C/N

The C/N ratio in the product ranged between 10.1 and 12.3. It was lowest in the Autoclaved treatment, followed by EM and highest for the Control (Table 10).

Table 10: C and N properties of the product, showing Total Nitrogen, Total Carbon and C to N ratio for EM, Autoclaved and Control treatment.

	EM Bokashi				Autoclaved				Control			
tot-N (%)	4.62	4.59	4.48	4.44	4.81	4.70	4.26	4.43	4.11	3.83	4.53	4.65
tot-C (%)	49.0	48.5	48.2	48.4	48.8	49.0	48.3	48.4	47.7	47.3	48.5	48.8
C/N	10.6	10.6	10.8	10.9	10.1	10.4	11.4	10.9	11.6	12.3	10.7	10.5

Calculations of C balance based on weight in relationship to measured loss varies (Table 11). The N balance show a lower loss if based on the mass of added kitchen waste and product, where the calculated values were negative in most cases (Table 12). One of the calculated C net loss was also negative. The mean calculated C loss/measured C loss were 53/21, 6/22, and 65/23 for EM Bokashi, Autoclaved and Control respectively. For N loss it was -23/1.8, -27/2.0 and -12/2.2. The variance was much higher for the calculated values than the measured.

Table 11: C balance comparing calculated C net loss and measured C loss. All values are expressed in mass (g).

	C_{start}	C_{end}	C net loss	CH_4-C tot	CO_2-C tot	$C_{leachate}$	$C\ loss_{measured}$
EM 1	2138	2044	**94.0**	1.03E-05	11.5	11.4	**23.0**
EM 2	2138	2125	**12.4**	1.01E-05	11.5	11.4	**22.9**
EM 3	2138	2095	**42.3**	7.46E-06	10.2	8.6	**18.8**
EM 4	2138	2073	**64.6**	7.81E-06	11.1	8.3	**19.4**
AC 1	2138	2176	**-38.2**	6.86E-06	11.2	9.6	**20.8**
AC 2	2138	2137	**0.3**	8.25E-06	12.3	14.7	**26.9**
AC 3	2138	2090	**47.2**	9.76E-06	10.1	9.6	**19.7**
AC 4	2138	2123	**14.2**	1.30E-05	10.4	10.5	**20.9**
C 1	2117	2060	**57.1**	7.91E-06	11.8	8.1	**20.0**
C 2	2117	1949	**167.3**	7.98E-06	11.6	15.2	**26.8**
C 3	2117	2087	**29.8**	6.93E-06	11.6	9.4	**21.0**
C 4	2117	2112	**5.0**	1.15E-05	10.5	15.2	**25.7**

Table 12: N balance comparing calculated N net loss and measured N loss. All values are expressed in mass (g).

	N start	N end	N net loss	N_2O-N total	N leachate	measured N loss
EM 1	172	193	**-21**	0.002	2.08	**2.09**
EM 2	172	201	**-29**	0.002	2.08	**2.09**
EM 3	172	195	**-23**	0.003	1.56	**1.57**
EM 4	172	190	**-18**	0.003	1.51	**1.51**
AC 1	172	214	**-42**	0.005	1.86	**1.86**
AC 2	172	205	**-33**	0.006	2.63	**2.64**
AC 3	172	184	**-12**	0.003	1.72	**1.72**
AC 4	172	194	**-22**	0.003	1.87	**1.88**
C 1	171	177	**-7**	0.005	1.48	**1.48**
C 2	171	158	**13**	0.005	2.77	**2.77**
C 3	171	195	**-24**	0.004	1.71	**1.71**
C 4	171	201	**-30**	0.003	2.77	**2.77**

4. Discussion

4.1. GHG emissions

The experiment did not show significant difference in total GHG emissions when adding EM Bokashi bran during the fermentation phase. However, the emissions are low compared to typical fluxes found in the compost studies. Considering that fermentation is used as preservation method, low emissions is expected in that step. But since this was true regardless of treatment, other factors such as pH, C/N and water content can be more relevant factors for reducing emissions.

N_2O

The N_2O emissions were high in the feeding phase, which fits well with the theory that fluctuating oxygen levels favour the coupled processes of nitrification and denitrification. Regular opening of the lid to add more kitchen waste creates favourable conditions for this.

Bokashiworld In Sweden AB recommends not to open the lid more than 1-2 times per day (*Bokashi,* 2018). They suggest that one should save the waste of each day and throw it all at the same time. Here the buckets were opened every third day, which might give different denitrification rates and N_2O emissions than normally for Bokashi.

In the resting phase, the N_2O levels were highest at the first measuring after 6 days and decreased exponentially thereafter. The continuously closed lid will eventually lead to anaerobic condition that reduces nitrification, leading to a limitation of nitrate for denitrification. Reduction of N_2O to N_2 can still proceed though. The share of N_2O emission from the resting phase to the total levels are almost negligible in this study. This can be explained by oxygen depletion.

No significant difference between the treatments were found in total emissions. However, a trend for lower emissions in the buckets treated with EM Bokashi was found. In the resting phase EM Bokashi did emit significantly lower amount of N_2O than the Autoclaved treatment, and for some of the measuring this was true compared to the Control as well. Quiroz & Céspedes (2019) have brought up a knowledge gap in understanding interactions between EM and native microflora such as organisms connected to the N cycle. In an earlier study an accumulation of NH_4^+ was measured in anaerobic systems, which was not seen in the leachate here.

CH_4

The addition of EM Bokashi did not have a significant impact on the total CH_4 emissions in this experiment. However, for the resting phase emissions were significantly lower than the other treatments. This did not affect the total emissions, since the highest mean emission over the feeding phase was from the EM treatment, and low emissions in general were detected.

The low emissions over the feeding phase are in line with the theoretic background. Methanogenesis is the fourth step of anaerobic digestion and methanogens are strictly anaerobes.

In the anaerobic resting phase, higher emissions were expected from the Control treatment at least. An initial increase was detected, but it never reached rates found in compost (Andersen et al. 2010 (0.25 g/h)). That might be explained by some factors that can have inhibit the methanogens. The acidic condition in all treatments is one. The relative low temperature, compared to both controlled environment for biogas production and compost, might also have an impact. However, the literature review gave one example of Bokashi treatment at controlled mesophilic temperature (37°C), and still no CH_4 was produced (Hanafiah 2017). High NH_3 levels is another factor brought up by Hanafiah (2017). In this study, a low C/N ratio, together with a 1.5-1.6 g/L NH_4^+ concentration in the leachate and relatively high N_2O-production suggest that it might have an influence.

The fact that the EM treatment still had significantly lower emissions during the resting phase, could be that it had the lowest pH. If the pH decreased over time, it might have reached a critical low level for methanogens in the EM treatment earlier, due to the lactic acid bacteria added. Acidity is known as an inhibitor for methanogenesis and for further knowledge pH could be measured continuously over time.

The results from the last sampling, show decrease of CH_4 to similar levels as the start values (just above air concentrations). Since this occurred in all buckets, leakage from them is not likely, and the GC measurements have been double checked. The N_2O levels was constantly decreasing suggesting that the O_2 depletion was intact. The CO_2 measurements showed an increase for the last measurement, which indicate that there was no leaching either. It exists anaerobic methanotrophs in nature but they are normally found in sediments (Bhattarai et al. 2019). All measurements from the third sampling were excluded and interpreted as outliers, when calculating the EF over the experiment. To include the third measurement

would not affect the above reasoning concerning low emissions over the experiment though.

CO_2

The total respiration over the fermentation did not differ between the treatments. In contrast to the other two GHGs, CO_2 emissions were highest during the resting phase from the buckets treated with EM. This is reasonable when considering the lower CH_4 levels. Methanogenesis both consumes and produces CO_2 at the same time and some of the C is emitted as CH_4. Other metabolic pathways may generate more net CO_2. Hanafiah (2017) measured values also supports the theory of high CO_2 with low CH_4. The addition of microorganisms can also increase the microbial activity, hence the respiration. With higher respiration rates the oxygen depletion occurs earlier in time and N_2O levels start to decrease.

All results from the CO_2 measuring were much higher the calibration levels for the GC, which means a higher uncertainty of the numbers.

C and N balance

The N balances show lower levels than measured, with mainly negative number. Both C and N balance from weight calculations have a higher variance that measured emissions and leachate loss. The results are probably due to underestimation of start levels, which were estimated from a table for the King Edward potatoes. There was also a time lap between the content analysis of potatoes and the start of the experiment. Visual signs of degradation in form of mould were found on the Gotland variety, before adding to the buckets. The added peel is also a factor.

The low or negative levels from the weight balance could however support the relative low emissions from all treatments.

4.2. Design of experiment

In some respects, this study might best be interpreted as a pilot. The method developed over the experiment, which makes some of the data difficult to compare. For example, the first measuring in the resting phase added some air to the buckets disturbing the anoxic conditions. Better start values could have been obtained with a more structured schedule. Setting up exact times for measuring and filling might have increased precisions further. The regression sampling used is better adapted for open system and was rejected as a method for estimating emissions over longer

periods, after comparisons with the values after three days. Continuously measuring of background levels could increase the reliability of the measuring further.

The substrate used for simulated kitchen waste would avoid the risk of CH_4 inhibition if it had a higher C/N ratio. The same variety of potatoes might have reduced some variation also, resulting in a more reliable nitrogen and carbon balance. Higher water content for the substrate could have facilitate pH-measuring continuously over the experiment.

4.3. GHG emissions compared with compost

This study only measured GHG emissions during the fermentation phase of EM Bokashi. The main part of decomposition is predicted to occur after this for EM Bokashi and the hypothesis is therefore that more GHGs will be emitted in that step. This study only compared EM Bokashi in a practical experiment with anaerobic digestion rather than composting.

The CH_4 total EF detected in the fermentation phase of this study are between 0.01-0.00001 % of the ranges presented in the compost studies over the whole composting process (see Table 1). However, this is regardless of EM being added or not. As mentioned before the pH at the end was lowest for the EM Bokashi treatment, but it was also low for the Autoclaved and Control treatments in this experiment (Table 7). The time aspect is also important since measurements in composts studies have been over year(s) in some cases (Amlinger et al. 2008; Andersen et al. 2010). Even with low fluxes the total amount can be high with a longer storing, such as over the winter season in a Swedish context. For as long as the fermented kitchen waste stays in the bucket, pH can be an affective inhibitor tough. The literature showed that CH_4 emission was negligible in a biogas study, explaining this both with pH and high levels of from ammonia and H_2S being toxic for methanogens (Hanafiah 2017). Biogas production is best compared with the fermentation phase. The more interesting question is what happens after the fermented product is covered by soil. A pH increase can mean a high risk for more CH_4 developing. It has already been shown that Bokashi gives a higher respiration rate at this stage (Shin et al. 2017; Thorslund 2020), with an increased risk of oxygen depletion. But other GHGs have not been measured.

For N_2O EF, the results are within the lower range of one home compost study (Table 1), already after the fermentation phase. That is worth noting, since the GWP of N_2O is high. Normally CH_4 is a bigger problem connected with organic waste treatment or composts, but in this study N_2O contributes more to global warming.

A factor that can explain the relatively high N_2O emissions is the C/N ratio (12.26) in the kitchen waste used in this study. This is lower than in the articles studying composts, which range 20-30. Amlinger et al. (2008) state that a C/N ratio below 17 can lead to higher N_2O emission in composts and recommend C/N-ratios between 25-35. The compost studies with low N_2O emissions used bulk agents (Yang et al. 2013) and controlled moisture content (Ermolaev et al. 2019) as mitigation strategies. The bulk agents gave high C/N ratios, while it was approximately 23 in the moisture study. The literature study also pointed out the risk of using N rich substrate. On the other hand, if the EFs in this study are compared with the highest levels from home composts though, they only reached 0.1-11 % of the whole composting process. It would therefore be interesting to determine how much N_2O is emitted from the mixing-with-soil-step of Bokashi. Important is also that the high emissions were found in all three treatments. It is probably the procedure of feeding N rich substrate to an environment with fluctuating oxygen that causes the emissions, not the adding of EM.

Short term results from the review showed higher respiration from soil samples (sandy) treated with EM Bokashi compared to a control treatment (Shin et al. 2017). From the theoretical perspective the main degradation occurs then, resulting in a postponed risk of GHG emission. Thorslund (2020) confirmed high respiration rates in a related study to this thesis. CO_2 was measured in an incubation study, after adding the three different treatments products with soil. This was compared with composted kitchen waste which had a significantly lower respiration than the other three treatments, with the largest difference during the first 8 days. On fields study detected higher CO_2 emissions compared to the control treatment (Mayer et al. 2010). The difference seem to decrease with time and for both (Mayer et al. 2010) and Shin et al. (2017) the effect was no longer significant after eight weeks. The high respiration also supports a need for measuring CH_4 and N_2O for that period as well. A short-term increase of respiration is not necessarily a bad thing, only if it affects the C balance. In Thorslund's (2020) study the net C loss was 66 %, and 70 % for EM Bokashi vs compost, so the total C sequestration may not differ as much as suggested by EM Agaton.

As suggested by Quiroz & Céspedes (2019), a better knowledge of interaction between EM and soil microorganisms might help understanding the risk for emissions after the fermentation. The sequencing of *R. palustris* genome show an ability of C and N fixation, but less is known about the potential in EM Bokashi.

Results from several studies are not included in this comparison due to the poorly estimated N content in the substrate used, showing a negative balance. Studies that give results as percentage of total N and ratios are therefore excluded. The

uncertainties of the CO_2 measurements are also a factor that excludes some compost comparisons.

Conclusion

1. There is insufficient information from the review to conclude whether GHG emissions from Bokashi and compost are similar. CH_4 and N_2O need to be measured over the whole production of Bokashi, especially after mixing the fermented substrate with soil.

2. Adding of EM bokashi bran does reduce CH_4 production during the resting phase in a significant way. But not in the feeding phase. The total amount emitted was not affected in this study. This is compared with anaerobic digestion.

3. Bokashi shows a high respiration rate and releases both CH_4 and N_2O.

This study does not support the branding of EM Bokashi as a more climate friendly alternative to compost in terms of GHGs. and falsify the statements of no CH_4 and low CO_2 emissions.

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Appendix I: Schedule for timeseries

Table 13: The time schedule used during the feeding phase 14th, 17th, 20th, 23rd and 26th of February.

	time (min:sec)	
	0:00:00	Lid 1 closed/Lid 2 opened
replica 1	0:00:30	Sampling start (bucket 1)
replica 1	0:01:00	Air the pump, switch to bucket 2
replica 1	0:02:00	Lid 2 closed/Lid 3 opened
replica 1	0:02:30	Sampling start (bucket 2)
replica 1	0:03:00	Air the pump, switch to bucket 3
replica 1	0:04:00	Lid 3 closed/Lid 4 opened
replica 1	0:04:30	Sampling start (bucket 3)
replica 1	0:05:00	Air the pump, switch to bucket 4
replica 1	0:06:00	Lid 4 closed/Lid 5 opened
replica 1	0:06:30	Sampling start (bucket 4)
replica 1	0:07:00	Air the pump, switch to bucket 5
replica 1	0:08:00	Lid 5 closed/Lid 6 opened
replica 1	0:08:30	Sampling start (bucket 5)
replica 1	0:09:00	Air the pump, switch to bucket 6
replica 1	0:10:00	Lid 6 closed
replica 1	0:10:30	Sampling start (bucket 6)
	0:12:00	Air the pump, switch to bucket 1
replica 2	0:12:30	Sampling start (bucket 1)
replica 2	0:14:00	Air the pump, switch to bucket 2
replica 2	0:14:30	Sampling start (bucket 2)
replica 2	0:16:00	Air the pump, switch to bucket 3
replica 2	0:16:30	Sampling start (bucket 3)
replica 2	0:18:00	Air the pump, switch to bucket 4
replica 2	0:18:30	Sampling start (bucket 4)
replica 2	0:20:00	Air the pump, switch to bucket 5
replica 2	0:20:30	Sampling start (bucket 5)
replica 2	0:22:00	Air the pump, switch to bucket 6
replica 2	0:22:30	Sampling start (bucket 6)
	0:24:00	Air the pump, switch to bucket 1
replica 3	0:24:30	Sampling start (bucket 1)
replica 3	0:26:00	Air the pump, switch to bucket 2
replica 3	0:26:30	Sampling start (bucket 2)
replica 3	0:28:00	Air the pump, switch to bucket 3
replica 3	0:28:30	Sampling start (bucket 3)
replica 3	0:30:00	Air the pump, switch to bucket 4
replica 3	0:30:30	Sampling start (bucket 4)
replica 3	0:32:00	Air the pump, switch to bucket 5
replica 3	0:32:30	Sampling start (bucket 5)
replica 3	0:34:00	Air the pump, switch to bucket 6
replica 3	0:34:30	Sampling start (bucket 6)
	0:36:00	Air the pump, switch to bucket 1
replica 4	0:36:30	Sampling start (bucket 1)
replica 4	0:38:00	Air the pump, switch to bucket 2
replica 4	0:38:30	Sampling start (bucket 2)
replica 4	0:40:00	Air the pump, switch to bucket 3
replica 4	0:40:30	Sampling start (bucket 3)
replica 4	0:42:00	Air the pump, switch to bucket 4
replica 4	0:42:30	Sampling start (bucket 4)
replica 4	0:44:00	Air the pump, switch to bucket 5
replica 4	0:44:30	Sampling start (bucket 5)
replica 4	0:46:00	Air the pump, switch to bucket 6
replica 4	0:46:30	Sampling start (bucket 6)
	0:48:00	End sampling