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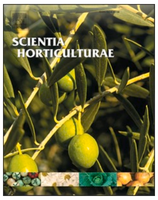

Nutrient utilization and growth of tomato crops fertilized with solid anaerobic digestate

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ABSTRACT

Anaerobic digestate is a valuable resource for horticultural production, as it contains nutrients and fibers that can be used in plant growing medium. However, compared with hydroponic production based on mineral fertilizers, obtaining accurate nutrient supply at each stage of the growth cycle may be challenging. In an experiment using container-grown tomato crops, we evaluated different fertilization regimes with solid anaerobic digestate (SAD). Four different treatments were compared, two involving different proportions (19 % and 37 % by volume) of SADs in the peat-based growing medium, one treatment where growing medium with 37 % SAD was inoculated with active nitrifying bacteria, and one treatment where 15 % (v/v) of the peat in growing medium with 37 % SAD was replaced with sawdust to control nitrogen (N) availability during cultivation. A mineral-fertilized treatment (N-P-K 5–1-5) with approximately similar N amount as in the treatment with 37 % SAD was used as reference. Nutrient availability, nutrient uptake efficiency, crop performance (plant growth, biomass accumulation), and plant stress (chlorophyll fluorescence) were monitored during cultivation. The concentration of ammonium was initially high (190–416 mg/L substrate) in the growing media fertilized with anaerobic digestate, while the concentration of nitrate was low. Readily available ammonium concentration decreased rapidly during cultivation, to around 50 % after 10 days and to almost 100 % by the end of the cultivation. Available nitrate concentration was initially low (0–8 mg/L in the different treatments) and decreased to zero within a week, but increased slightly from day 40 of cultivation. Nutrient use efficiency was generally higher (15–50 % for different nutrients) in the treatment with 19 % digestate. Inclusion of sawdust in the growing medium decreased nutrient use efficiency by 30–50 %. Compared with the mineral-fertilizer reference, biomass production was lower in all treatments fertilized with digestate, with 37 % and 19 % SAD resulting in 62 % and 47 % of total biomass obtained in the reference, and similar reductions in yield of harvestable fruits. Chlorophyll fluorescence measurements indicated elevated plant stress in the treatments fertilized with SAD. Addition of sawdust or nitrifying bacteria did not help to control nitrogen availability during cultivation. Therefore, anaerobic digestate fertilizers need further optimization before they can be a competitive alternative to mineral fertilizers.

1. Introduction

One way to increase sustainability in food production is to recirculate organic waste as a resource-efficient fertilizer in growing substrates, e.g., in the horticultural sector, since closing the gap between production and consumption requires recirculation of food waste, crop residues, and other agricultural wastes (Reganold and Wachter, 2016; Tittarelli, 2020). Anaerobic digestion of organic residues in biogas reactors produces renewable energy and a residue (digestate) containing nutrients essential to plant growth (Bergstrand, 2022). This anaerobic digestate, which comprises a slurry of water and organic residues, has been shown to have useful chemical and physical characteristics for the horticulture industry (Kumar et al., 2022). It can be used as a plant fertilizer directly or after further processing, thereby contributing to closing global energy and nutrient cycles (Alburquerque et al., 2012; Möller and Müller, 2012).

Anaerobic digestate has been successfully used as a fertilizer for several horticultural crops in soilless systems, and in unfertilized peat-based growing media (Cheong et al., 2020; Pitts, 2019). Studies on hydroponic systems include e.g., lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and pak choi (*Brassica rapa*) (Kamthunzi, 2015; Neal and Wilkie, 2014; Pelayo Lind et al., 2020).

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To increase the usefulness of the digestate, it can be separated into a solid fraction (solid anaerobic digestate, SAD) and a liquid fraction, providing the possibility for use as a liquid fertilizer and growing substrate. However, during separation, nitrogen (N) and phosphorus (P) typically end up in the liquid fraction. For example, a year-long study on screw press solid-liquid separation of digestate from 13 full-scale digestion plants found that 87 % of total N and 71 % of total P were in the liquid fraction and the remainder in the solid fraction (Tambone et al., 2017). Another study comparing six anaerobic digestates before and after solid/liquid separation found that about 20 % of total N and 35 % of total P ended up in the solid fraction (Mazzini et al., 2020). SAD has recently been used as a growing substrate and sole fertilizer in trials with tomatoes and basil (*Ocimum basilicum*) (Bergstrand et al., 2020; Asp-et al., 2022).

Due to the unequal separation of nutrients between the solid and liquid fractions, it is often beneficial to use both fractions in crop production. A combination of the two fractions has been tested for crops with relative short culture time, such as basil, peppermint (*Mentha* × *piperita*), lettuce (Ronga et al., 2019) and parsley (*Petroselinum crispum*) (Pokhrel et al., 2018). In a similar study with a longer growing period, Stoknes et al., (2018) obtained good yields of tomatoes using digestate derived from source-separated food waste and animal manure in a cropping system where the solid parts of the digestate (NH_4—N:P:K ratio of 1.4:1:1.1) were vermicomposted and used as growing media together with green waste compost. The unseparated digestate (NH_4—N:P:K ratio of 6:1:5) was used as a fertilizer in a recirculating system with an integrated nitrification biofilter, since it was found that using the liquid phase alone resulted in P-deficiency in tomato plants (Stoknes et al., 2018). Yields in the digestate treatment were found to be similar to those obtained with a synthetic mineral fertilizer in peat-based substrate (Stoknes et al., 2018).

Within the European Union (EU), greenhouse production of e.g., vegetable transplants, herbs, and pot plants uses peat as the main substrate (Schmilewski, 2009), but negative environmental aspects of peat extraction provide strong incentives to use alternative horticultural substrates (Ceglie et al., 2015). Due to its favorable physical, chemical, and growth-promoting traits, SAD is an interesting candidate as a peat substitute. However, replacing a larger proportion of peat-based growth medium with SAD can have negative consequences for plants, such as high ammonium:nitrate ratio, high pH, and high electric conductivity (EC), decreasing the benefits of SAD (Asp-et al., 2022). Rectifying these problems using additives is complicated when aiming to produce a certified organic produce, since EU legislation EU (EG 834/2007) requires a substantial proportion of plant nutrients to be supplied from the start of cultivation.

In a previous study on a tomato crop, Bergstrand et al., (2020) tested a peat- and SAD-based substrate containing enough mineralized N for around four months of growth and harvesting and found that SAD performed well compared with other organic fertilizers. However, there were indications that high ammonium (NH_4) concentration at the beginning of the study hampered plant growth. Phytotoxic effects of NH_4 and also of ammonia (NH_3) have been reported for a large range of plant species (Britto and Kronzucker, 2002; Pan et al., 2016). Nitrification, i.e., enzymatic conversion of NH_4 to nitrate (NO_3) by bacteria, can be promoted to decrease the NH_4 concentration in substrate and increase the NO_3 concentration (Stoknes et al., 2018). Nitrification can also occur without any active effort to introduce nitrifying bacteria, as in the study by Bergstrand et al., (2020). The high NH_4 concentration in anaerobic digestate originates from degradation of proteins in the biogas reactor. The non-mineralized N remaining after anaerobic digestion is bound in relatively stable organic compounds, with reported net mineralization rate of 8 % and 12 % of organic N during the first three and six months, respectively, after soil application (Moorhead et al., 1987; Gunnarsson et al., 2010). The C/N ratio in the residue affects the mineralization rate, where total carbon (C) content normally varies between 28 % and 47 % of dry matter (Möller, 2015). Thus in combination, mineralization, nitrification, and plant uptake govern the NH_4 concentration in growing substrate.

The present study investigated whether a single application of SAD, without any secondary fertilization, in tomato cultivation can supply sufficient nutrients for a prolonged growing period. Mineralized N content was monitored throughout cultivation and two potential ways of altering the N mineralization and nitrification (altering the C/N ratio and inoculation with active nitrification bacteria) were tested. Concentrations of nutrients in tomato shoots and fruits and macronutrient uptake efficiency were also assessed.

2. Materials and methods

2.1. Growing conditions, plant material, and growing media

Tomato cultivation was performed in a 90 m^2 greenhouse chamber at the Swedish University of Agricultural Sciences, campus Alnarp (55°N), Sweden. The climate set points were 18 °C for heating and 21 °C for ventilation via rooftop vents. Shading screens were set to close when the outside radiation exceeded 700 W m^{-2}. The climate was controlled by a greenhouse computer (Priva Compact, Priva, de Lier, the Netherlands). The growing period (from sowing to harvest) ran between January 10 and May 17. Additional light was provided by 400 W high-pressure sodium lamps (Philips, Eindhoven, the Netherlands) from January 10 to March 20, after which only natural light was provided.

Tomato seeds (*Solanum lycopersicum* cv. Torelino, Olssons frö, Helsingborg, Sweden) were sown in organic certified sowing substrate (Simontorp ekologisk såjord, Weibulls, Åby, Sweden). After 10 days, the plantlets were transferred to organic certified planting soil (Hasselfors EKO, Hasselfors Garden AB, Örebro, Sweden), where they were kept for two weeks before being planted in the final substrate with different treatments as shown in Table 1. Twenty-five plants (five per treatment) were planted in 30 L substrate in 45 L pots on trays that were randomly distributed on the greenhouse floor.

The growing substrate used was based on peat moss (0–25 mm, H2-H7, SW Horto AB, Hammenhög, Sweden) and dewatered anaerobic digestate (SAD) from a commercial biogas producer (Gasum AB, Jordberga, Sweden). The feedstock for the digestate comprised (percent by weight): water 34 %, recirculated digestate 30 %, by-products from food industry 18 %, crop residues and other vegetable byproducts 18 %, and iron chloride 0.4 %. The digestate was dewatered by a screw press to final dry weight (DW) of 27 %. The substrate in one treatment with 37 % SAD included sawdust (1–3 mm) from alder (*Alnus glutinosa*) with C/N ratio 214). The reference treatment (see Table 1) was fertilized with a two-component mineral fertilizer consisting of Calcinit™ and Kristalon™ Indigo (Yara, Oslo, Norway), with 10 g of each component per L

Table 1
Substrate mixes used for tomato cultivation. The values refer to the amount in each of five pots per treatment. The percentages are volume-based. Solid anaerobic digestate (SAD) was mixed with peat to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.).

Treatment	Peat, %	SAD, %	Sawd. %	Lime, g	Fertilizer, L *	Inoc., L**
Mineral ref.	100	0	0	312	8.8	0
SAD 37 %	63	37	0	132	0	0
SAD 37 % Inoc.	63	37	0	132	0	2
SAD 19 %	81	19	0	180	0	0
SAD 37 % Sawd.	48	37	15	94	0	0

* Liquid fertilizer given as 300 mL per pot twice a week.

** Liquid anaerobic digestate per pot given in the beginning of the growing period.

solution. One treatment with 37 % SAD was inoculated with liquid anaerobic digestate, of the same origin as the SAD, containing active nitrification bacteria cultivated in liquid anaerobic digestate under greenhouse conditions in our laboratory for just over six years. Mean conversion rate of NH_4^+ to NO_3^- in the aerated liquid digestate was 10 g N m^{-3} d^{-1}.

2.2. Treatments and plant management

The study consisted of five treatments (Table 1). Peat, SAD (37 % or 19 %), and sawdust (when included) were weighed to the desired proportion (v/v) in terms of bulk density, and thoroughly mixed. The mineral reference treatment was fed with a mineral-based nutrient solution each week, while the other treatments were compensated for this addition with tap water. The total amount of N in the mineral-based nutrient solution was equal to the N content in the 37 % SAD treatment, which was designed to provide each plant with 30 g N, based on Bergstrand et al., (2020). The N concentration in SAD was 0.79 % (FW basis). Lime (agricultural grade $Ca(CO_3)_2$) was used to adjust the pH to 6.5, after a pre-trial with different lime additions. Different amounts of lime were needed in the different treatments due to the alkaline nature of SAD (pH 8.3) (Table 1). The substrates in each pot were mixed and watered one week prior to planting.

The plants were watered separately using CNL ND drip irrigation (Netafim, Tel Aviv, Israel), with two irrigation cycles daily. The duration of the irrigation cycles was adjusted to reach field capacity without draining. Differences depending on treatments were adjusted by manual irrigation. No water or nutrients were lost through drainage, due to the trays under the pots. To prevent root diseases, 1 g/pot of *Trichoderma harzianum* T-22 (Trianum, Koppert, Berkel en Rodenrijs, the Netherlands) was added as a suspension to the substrate before planting. To prevent sciarid flies, *Bacillus thuringiensis* (Gnatrol, Nordisk Alkali, Malmö, Sweden) was added to the pots, as a liquid suspension, on two occasions during the experiment.

The plants were kept upright by winding the stems around wire suspended from the greenhouse roof. Side-shoots were trimmed off weekly and the plants were defoliated from below to maintain approximately 21 mature leaves on the stem. All harvested plant material was dried and weighed separately for each plant.

2.3. Measurements and analyses

2.3.1. Growing substrates

The pH and EC of the growing substrates were determined according to European standard EN13038:201, where one part growing medium was extracted in five parts distilled water. Dry bulk density was determined according to European standard EN13040:2007), where approximately 1 L of growing medium was compacted by a 634 g weight in a steel cylinder. The exact volume of the compressed substrate was determined and used to calculate dry bulk density. Compact density of the material (density without pores) was determined by adding a defined amount of the growing medium (approximately 5 g) to a 50 mL volumetric flask together with 25 mL 96 % ethanol, shaking for 30 min and then topping the flask up to the 50-mL mark with alcohol. The total added volume of ethanol was measured and, by difference, the volume of the substrate, and compact density was calculated as sample weight divided by sample volume. Porosity of the growing substrate was calculated as:

$$Porosity\ (\%) = (1\text{-(Bulk density/Compact density) x 100)} \quad (1)$$

Water-filled pore space after drainage was determined by filling a plastic cylinder (0.75 L) with the substrate. The cylinder was perforated at the base, allowing water to drain out, and had a removable collar on the top. The cylinder and collar were filled with substrate and compacted by a 634 g weight for three minutes. The cylinder was then immersed in water for two days, to allow it to become totally water saturated, and left on a steel grid to drain for three days, during which the top of the cylinder was covered with plastic film to prevent evaporation. After three days of drainage, the collar was removed and excess substrate was carefully removed to give a final volume of 0.75 L. The substrate was weighed, dried for four days at 105 °C, and dry weight was determined. The volume of water in each sample was compared against the total porosity and the volume of the water-filled pores was calculated.

Readily-available plant nutrient levels were estimated by the modified Spurway-Lawton (SL) extraction procedure (Spurway and Lawton, 1949), the most used method for determination of fertilizer demand by Swedish greenhouse growers. For this, 25 mL of substrate were extracted for 30 min in an end-over-end shaker with 150 mL acetic acid (0.018 mol L^{-1}), filtered, and analyzed. These extractions and analyses were performed by a commercial agricultural laboratory (LMI AB, Helsingborg, Sweden). Total concentrations of nutrients in the substrate were determined by extraction in concentrated nitric acid under pressure using the microwave technique, followed by analysis by ICP-OES according to Swedish standard SS 28,311:2017 performed by the same commercial laboratory.

2.3.2. Plants

Tomato fruits were harvested when ripe, beginning 60 days after planting in the treatment substrates. Cumulative weight was recorded and total yield was calculated (FW and DW). At the end of the growing period, unripe fruits were harvested.

Chlorophyll fluorescence (F_0, F_m, F_v/F_m) was measured on five occasions during the growing period (see Fig. 2), using a PAM-2500 (Heinz Walz GmbH, Effeltrich, Germany), after 20 min of dark adaptation of the leaf. The chlorophyll content in leaves was determined 10 days before harvest, using a MC-100 Chlorophyll Meter (Apogee Instruments, North Logan UT, USA).

At the final harvest, fruits, stems, and leaves were harvested, and FW and DW (dried at 70 °C for seven days) were determined. Dry weight of previously harvested side-shoots and leaves was added to the DW value. A sample consisting of a stem piece (30 cm) and five leaves per plant was milled and nutrient concentrations were analyzed. A sample consisting of fruits (10 per sample) from each plant was analyzed in the same way. These analyses were performed by an accredited laboratory using ICP-OES (LMI AB, Helsingborg, Sweden).

2.3.3. Statistical analysis

The trial was a completely random design with five treatments and five replicates per treatment ($n = 5$). One-way analysis of variance (ANOVA) and Tukey's HSD test for differences of means, with confidence interval set to 95 %, were used for statistical analysis of the data obtained from the experiment. The software used was Minitab Express version 17.

3. Results

3.1. Physical and chemical properties of the substrate

Bulk density increased and porosity of the substrate decreased on inclusion of SAD as a substrate component, compared with the mineral reference based on 100 % peat (Table 2). With 37 % SAD, the porosity decreased by 4–7 % and the volume of water-filled pores increased by 4–10 %, resulting in approximately similar water-holding capacity but with a smaller percentage of air-filled pores. With 19 % SAD, porosity and volume of air-filled pores remained the same as in the reference treatment (Table 2). The treatments with 37 % SAD non-inoculated and inoculated with active nitrifying bacteria had the highest available concentrations of NH_4, P, and potassium (K) at the beginning of the experiment (analyzed by the SL method) (Table 3). The 19 % SAD treatment had 53 % less P and K compared with the 37 % SAD treatment

Table 2
Physical properties of the substrates at the start of the experiment. Solid anaerobic digestate (SAD) was mixed with peat to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). Different letters denote significant differences at $p<0.05$ (Tukey, $n = 3$).

Treatment	Bulk density, g L^{-1}	Porosity,%	Water-filled pores,%
Mineral ref.	218 b	83 a	64 b
SAD 37 %	276 a	79 b	68 ab
SAD 37 % Inoc.	300 a	76 c	74 a
SAD 19 %	218 b	83 a	67 b
SAD 37 % Sawd.	284 a	77 bc	66 b

and 62 % lower NH_4 content. Note that the mineral reference treatment (given fertilizer solution weekly) is not included in the statistical analyses in Tables 3-5, which refer to the SAD treatments where all the nutrients were present from the beginning of the study. Inoculation of the 37 % SAD treatment with active nitrifying bacteria did not change the availability of any nutrient, except for a minor change for copper (Cu). Incorporation of sawdust in the substrate reduced NH_4 availability by around 50 %. The concentration of NO_3 was low (<10 mg L^{-1}) at the start of the experiment for all treatments (Table 3). The sodium (Na) concentration exceeded 100 mg L^{-1} for the treatments with 37 % SAD, while due to dilution effects it was lower in the treatments with 19 % SAD and with sawdust inclusion (Table 3). Availability of P, K, sulfur (S), boron (B), iron (Fe), and zinc (Zn) was also significantly lower in the treatment with 19 % SAD and the treatment with sawdust compared with the treatments with 37 % SAD (Table 3). Almost no readily available N was present in the substrates at harvest, and large decreases were also seen for the macronutrients P, K, and S, while availability increased for magnesium (Mg) and Ca.

Treatments with 37 % SAD had significantly higher total concentrations of N, P, and K than the treatment with 19 % SAD and the sawdust treatment (Table 4). The C/N ratio was 30 for the substrates with 37 % SAD and 40 for the substrate with 37 % SAD in which part of the peat was replaced with sawdust (Table 4). The C/N ratio remained constant during cultivation in the 37 % SAD treatment, but decreased from 40 to 26 during cultivation in the sawdust treatment (data not shown).

3.2. *Nutrient availability over time*

Substrate concentration of NH_4 was initially high (190–416 mg L^{-1}) (Table 3, Fig. 1), but dropped quite rapidly after the beginning of the experiment in all treatments. The sawdust treatment lost 85 % of available NH_4 while the 19 % SAD treatment lost 40 %. The concentration of NH_4 remained lowest in the sawdust treatment throughout the experiment, while at 80 days after planting (DAP), the concentration was close to zero in all treatments (Fig. 1). Substrate concentration of NO_3 dropped to zero by 7 DAP, but at the end of the experiment (between day 40 and 80) it started to rise, indicating active nitrification. No NO_3 was detected in the 19 % SAD treatment until 80 DAP. Significant differences in NH_4 concentrations were only found at the first two measuring occasions. The concentrations of readily available P, K, S, B, and Fe also decreased during the experiment in all treatments (Table 3).

Table 3
Readily available nutrients, mg/L (Spurway-Lawton extraction), in solid digestate (SAD) mixed with peat to 37 % or 19 % (v/v), sampled (a) at planting and (b) after 14 weeks of tomato cultivation. One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). The mineral reference consisted of limed peat fertilized with mineral nutrients given in portions throughout cultivation. The reference was not included in the statistical analysis. Different letters denote significant differences at $p<0.05$ (Tukey, $n = 5$). No letter means no significant differences within the column. (n.d. = not detectable).

a) **at planting**

Treatment	pH	Nmin	NO_3–N	NH_4–N	P	K	Mg	S	Ca	Mn	B	Cu	Fe	Zn	Na
Mineral ref.	5.7	214	192	20	38	240	218	65	1220	2.12	0.28	0.44	2.34	1.24	27
SAD 37 %	6.8 a	422 a	8 a	416 a	236 a	500 a	132 a	43 a	384 b	1.58	0.76 a	0.68 a	1.3 a	2.9 a	113 a
SAD 37 % Inoc.	6.8 a	416 a	5 a	410 a	254 a	508 a	148 a	44 a	452 ab	1.42	0.82 a	0.49 b	1.4 a	3.0 a	128 a
SAD 19 %	6.2 b	256 a	n.d.	256 b	130 b	274 c	136 a	29b	534 a	1.38	0.4 c	0.44 b	0.5 b	1.7 b	75 b
SAD 37 % Sawd.	6.7 a	190 c	n.d.	190 c	144 b	372 b	110 b	26 b	342 b	1.16	0.58 b	0.42 b	0.8 b	1.9 b	86 b

b) at harvest

Treatment	pH	Nmin	NO_3–N	NH_4–N	P	K	Mg	S	Ca	Mn	B	Cu	Fe	Zn	Na
Mineral ref.	6.7	10.74	8.04	2.8	18.6	41.2	142	21.8	1300	1.44	n.d.	1.73	1.11	29.9	127
SAD 37 %	5.8	7.4	0.9	6.4 ab	86 ab	30 b	182	6.8	652	0.69	n.d.	1.17	0.58	6.4	175
SAD 37 % Inoc.	5.3	8	3.3	5.0 ab	99 ab	43 b	186	8.4	686	0.60	n.d.	0.46	0.52	7.0	156
SAD 19 %	6.1	3.2	0.7	2.5 b	34 b	15 b	175	6.8	846	12.35	n.d.	1.08	0.33	23.0	115
SAD 37 % Sawd.	6.0	12.5	2	10.6 a	123 a	208 a	188	8.2	590	0.71	n.d.	0.75	0.57	7.9	125

Table 4
Total mineral concentration, mg L^{-1}, in solid digestate (SAD) mixed with peat to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). The mineral reference consisted of limed peat fertilized with mineral nutrients given in portions weekly throughout cultivation. The reference was not included in the statistical analysis. Different letters denote significant differences at $p<0.05$ (Tukey, $p<0.05$, $n = 5$). No letter means no significant differences within the column.

Treatment	N_{tot}	P	K	Mg	S	Ca	Mn	B	Cu	Fe	Zn	Mo	Na	Ni	C	C/N
Mineral ref.	11,980	531	2868	6256	1968	54,800	67.2	12 b	6.7	1592	7.14	1.8	1411	0.75		
SAD 37 %	15,720 a	2610 a	4840 a	4314 ab	2010 a	26,900 bc	59 a	22 a	7.9 a	2328 a	20 b	1.5 a	1814 a	0.90	464,400	30 b
SAD 37 % Inoc	15,320 a	2806 a	4662 ab	4922 a	2000 ab	31,480 ab	64 a	21 a	7.3 a	2392 a	23 a	1.2 b	1482 ab	0.73		
SAD 19 %	13,180 b	1470 b	2548 c	4758 a	1888 b	37,880 a	59 a	13 b	6.4 ab	1834 b	12 d	1.1 bc	1028 b	1.11		
SAD 37 % Sawd.	12,140 b	1882 b	3930 b	3430 b	1338 c	20,580 c	41 b	19 a	5.5 b	1388 c	16 c	0.86 c	1333 ab	0.78	485,800	40 a

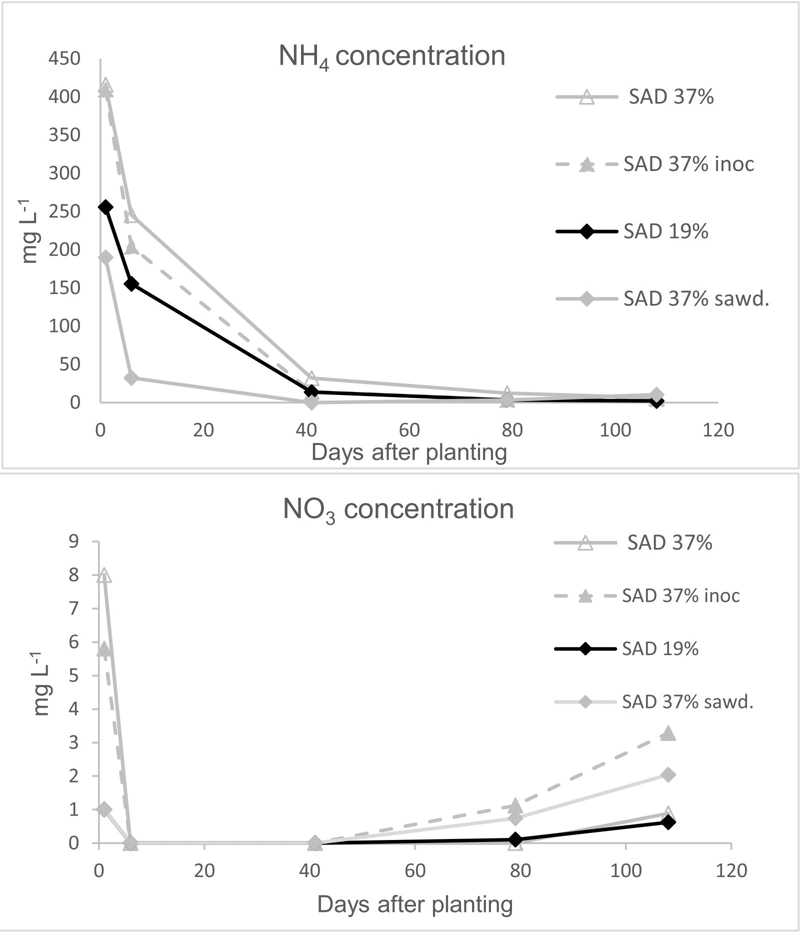

Fig. 1. Concentrations over time (DAP = days after planting) of (top panel) readily available ammonium (NH_4) and (bottom panel) nitrate (NO_3) in peat-based substrate mixed with solid anaerobic digestate (SAD) to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). .

However, the concentrations of Mg, Ca, Cu, Zn, and Na increased during the experiment in the treatments with SAD (Table 3).

Nitrogen uptake efficiency was in general low (12–34 %) for the treatments fertilized with SAD compared with the mineral reference (76 %) (Table 5). For the treatment with only 19 % SAD added, the uptake efficiency of macronutrients was generally higher than for the other treatments fertilized with SAD, with the exemption of Ca uptake efficiency. For N, P, Mg, and S, nutrient uptake efficiency was lowest in the treatment with sawdust (Table 5).

3.3. *Plant and harvest data*

In treatments with 19 % SAD or 37 % SAD and with addition of sawdust, tomato fruit ripened one week earlier than in the other treatments. The mineral reference treatment produced by far the highest tomato yield and biomass. Stem length measurements throughout cultivation showed that the mineral reference and the treatment with added sawdust had the tallest plants (Table 6). The treatments with 37 % SAD alone and 37 % SAD plus inoculation with active nitrification

Table 5

Macro-nutrient uptake efficiency (NUE, etc.), calculated as total content of minerals in aboveground plant parts (leaves, stem, and fruits) divided by the amount of minerals added by fertilization and liming in the treatments with solid digestate (SAD) mixed with peat and to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). The mineral reference consisted of limed peat fertilized with mineral nutrients given in portions weekly throughout cultivation. The reference was not included in the statistical analysis. Different letters denote significant differences at $p < 0.05$ (Tukey, $p<0.05$, $n = 5$). No letter means no significant differences within the column.

Treatment	NUE%	PUE%	KUE%	MgUE%	SUE%	CaUE%
Mineral ref.	76	58	58	8.2	81	20
SAD 37%	22 b	42 b	61 bc	57 b	19 b	12 b
SAD 37% Inoc.	18 b	42 b	72 ab	57 b	22 b	14 a
SAD 19 %	34 a	59 a	80 a	95 a	32 a	9.3 c
SAD 37 % Sawd.	12 c	22 c	51 c	36 c	9.6 c	8.5 c

Table 6

Growth and physical parameters of tomato plants grown in peat-based substrate mixed with solid anaerobic digestate (SAD) to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). The mineral reference consisted of limed peat fertilized with mineral nutrients given in weekly portions throughout cultivation. Length refers to final stem length. Different letters denote significant differences at $p<0.05$ (Tukey, $p<0.05$, $n = 5$). No letter means no significant differences within the column.

Treatment	Fruit FW, g	Fruit DW, g	Shoot DW, g	Total DW, g	Length, cm	Chlorophyll
Mineral ref.	3428 a	295 a	368 a	664 a	314 a	39.9 a
SAD 37 %	2385 b	236 b	186 b	423 b	270 b	16.3 b
SAD 37 % Inoc.	2251 b	222 b	182 b	404 b	266 b	11.7 b
SAD 19 %	1737 c	179 c	131 c	310 c	234 c	13.0 b
SAD 37 % Sawd.	1558 c	144 d	99 c	244 d	302 a	11.8 b

bacteria produced significantly higher fruit yield and total biomass than the treatments with 19 % SAD and 37 % SAD plus sawdust (Table 6). Using 50 % less SAD in substrate (19 % SAD compared with 37 %) decreased biomass production and tomato yield by approximately 25 %. Addition of sawdust to the substrate caused lower nutrient availability and lower nutrient uptake efficiency (Table 3A, Table 5) and also decreased all growth parameters measured (Table 6). Inoculation with active nitrification bacteria did not change any of the growth parameters compared with treatments with the same fertilizer, but without inoculation. The concentrations of N and S in tomato fruits were higher in the mineral reference treatment than in the treatments fertilized with SAD (Table 7). For P and K, concentrations in the fruits were highest in the treatment with sawdust (Table 7).

Leaf chlorophyll concentration (CCI) was 60 % higher in the mineral reference treatment than in the other treatments, which did not differ from each other. However, leaf chlorophyll concentration was only measured once, while chlorophyll fluorescence was measured on five occasions. The chlorophyll fluorescence (F_v/F_m) values were similar for all treatments at the beginning of the experiment, but at 41 DAP (March 23) F_v/F_m was significantly lower in the treatment with 19 % SAD. At 75 DAP (April 26), F_v/F_m was lower in all treatments with SAD than in the reference treatment (Fig. 2).

4. Discussion

Producing biogas by anaerobic digestion can increase sustainability in the food production chain in multiple ways. The biogas produced (biomethane) can be used as fuel for vehicles, for electricity generation, or for heating of e.g., greenhouses. Carbon dioxide, which can serve as a gaseous fertilizer in the greenhouse atmosphere, is also produced in the process. The residual slurry (digestate) contains minerals essential for plant growth and can be utilized directly in field production or, more appropriately for horticultural production, separated into a liquid and a solid fraction. The liquid fraction can be added as a fertilizer to nutrient solution used in drip irrigation or in hydroponics (Pelayo Lind et al., 2020), while the solid fraction can be used as a nutrient-rich constituent of growing media, potentially reducing peat use (Asp-et al., 2022). Different types of organic wastes can be used as feedstock for anaerobic digestion, so the process also contributes to waste management.

Artificial growing media and nutrient solutions can be tailored to match actual plant need, i.e., uptake and/or tissue concentrations of various elements (e.g., Sonneveld et al., 1999; Ingestad and Ågren, 1995). Similar adjustment is seldom possible when dealing with organic fertilizers with variable nutrient availability. Compared with eight commercially available growing substrates, the mix with peat and SAD used in the present study was found to have high availability of all macronutrients except for S and Ca, and to have low availability of all micronutrients except B, measured as readily available minerals by SL extraction (Schüssler and Bergstrand, 2011). In a comparable study, Nesse et al., (2019) found that in substrate with 20 % SAD, all macronutrients and several micronutrients were present in concentrations suitable for lettuce and tomato production. However, total concentrations of minerals were analyzed in that study, while available nutrients are often predicted by different extraction procedures, like the SL method used here or the more recent European standard method for growing substrates (CAT-extraction (SS-EN 13,651)). In this study, each of the macronutrients extracted with the SL procedure was significantly correlated with total uptake of the nutrient in aboveground plant parts (fitted line plot regression, Minitab version 17, data not shown). However, in this particular case total nutrient concentrations correlated well with plant uptake and SL extraction values correlated well with total nutrient concentration in the growing substrate. Thus mineralization of the organic substance seemed to release all macronutrients in similar manner, which may not be the case in a natural mineral-based soil.

Total element analysis of the SAD (Table 4) revealed high potential for use as a plant fertilizer. However, the usability of any organic residue as fertilizer depends on whether the nutrients are readily available to plants, i.e., mineralized and loosely bound to the substrate or dissolved in the soil solution. It has been shown the SAD can provide sufficient nutrient concentrations in mixes with peat for short-season crops, e.g. tomato, pepper (*Capsicum annuum*), musk melon (*Cucumis melo*) (Restrepo et al., 2013) and basil (Asp-et al., 2022). However, other studies with short-season crops, e.g., parsley, have found that fertilization with SAD requires amendment with additional sources of nutrients to support full crop development (Pokherel et al., 2018). A study by Stoknes et al., (2018) found that a tomato crop (long crop) fertilized with anaerobic digestate (SAD) developed well and was as productive as a conventionally fertilized crop when the SAD was combined with additional fertilization from the liquid phase of the digestate. The organic residue remaining after anaerobic digestion is rather recalcitrant to further mineralization compared with the feedstock of the digester, due to the degradation of more labile organic fractions during biodigestion (Tambone et al., 2019). However, the remaining organic N can mineralize at a rate of 8–12 % in the first three months (Moorhead et al., 1987; Gunnarsson et al., 2010) and can thus contribute to total N availability to plants during a longer cultivation period.

All essential plant nutrients should be present and available in growing medium in amounts high enough to support potential growth. The total content and availability are of interest, but the balance of nutrients also affects uptake efficiency and growth (Ingestad and Ågren, 1995). Nutrient availability in fertilizer that matches the actual demand of plants promotes greater growth of vegetative plant parts (Ingestad

Table 7

Concentrations of macronutrients (% dry matter) and micronutrients (mg/kg dry matter) in fruits and shoot parts of tomato plants grown in peat-based substrate mixed with solid anaerobic digestate (SAD) to 37 % or 19 % (v/v). One treatment was inoculated (Inoc.) with liquid anaerobic digestate containing active nitrification bacteria. One treatment had 25 % of the peat replaced with sawdust (Sawd.). The mineral reference consisted of limed peat fertilized with mineral nutrients given in weekly portions throughout the cultivation. Different letters denote significant differences at $p<0.05$ (Tukey, $p<0.05$, $n = 5$). No letter means no significant differences within the column .

Treatment	Ntot	P	K	Mg%	S	Ca	Mn	B	Cu	Fe	Zn Mf/Kg	Mo	Na	Ai	Si
Fruits															
Mineral ref.	2.25 a	0.42 b	2.38 ab	0.11 b	0.14 a	0.07 a	11.6 b	10.6 c	2.5	44.0 a	23.0 a	0.93 a	437	13.4	31.8
SAD 37 %	1.47 b	0.41 b	2.03 b	0.11 b	0.09 b	0.05 b	11.8 b	12.4 b	2.5	25.7 bc	14.0 b	0.46 b	403	14.1	29.8
SAD 37 % inoc	1.42 b	0.43 b	2.67 ab	0.12 b	0.10 b	0.05 b	12.8 b	13.7 b	2.7	28.1 b	15.0 b	0.37 b	427	16	32.2
SAD 19 %	1.40 b	0.40 b	2.19 b	0.10 b	0.10 b	0.06 ab	13.7 ab	12.7 a	2.5	23.6 bc	13.1 b	0.5 ab	419	15.9	32.6
SAD 37 % Sawd	1.32 b	0.47 a	3.21 a	0.13 a	0.09 b	0.08 a	15.9 a	14.1 a	2	22.8 c	12.9 b	0.28 b	462	13.4	31.4
Leaves and stems															
Mineral ref.	2.53 a	0.34 c	1.51 b	0.85 c	0.99 a	3.78 b	166 c	49 b	1.7	87 a	17 bc	1.2 a	1270 a	10	54 b
SAD 37 %	1.62 b	0.90 ab	1.69 ab	0.91 bc	0.27 b	3.78 b	337 b	88 a	1.5	52 b	19 b	0.3 bc	1398 a	11	62 ab
SAD 37 % Inoc.	1.63 b	0.95 a	1.83 ab	0.91 bc	0.33b	4.52 ab	415 a	103 a	1.7	49 b	16 bc	0.25 c	1254 a	11	67 ab
SAD 19 %	1.53 b	0.89 ab	0.92 c	1.10 a	0.34 b	5.35 a	422 a	101 a	1.5	41 b	13 c	0.61abc	1224 a	12	72 a
SAD 37 % Sawd	1.82 b	0.70 b	1.91 a	1.03 ab	0.24 b	5.06 a	436 a	101 a	1.5	52 b	25 a	0.97 ab	800 b	10	61 ab

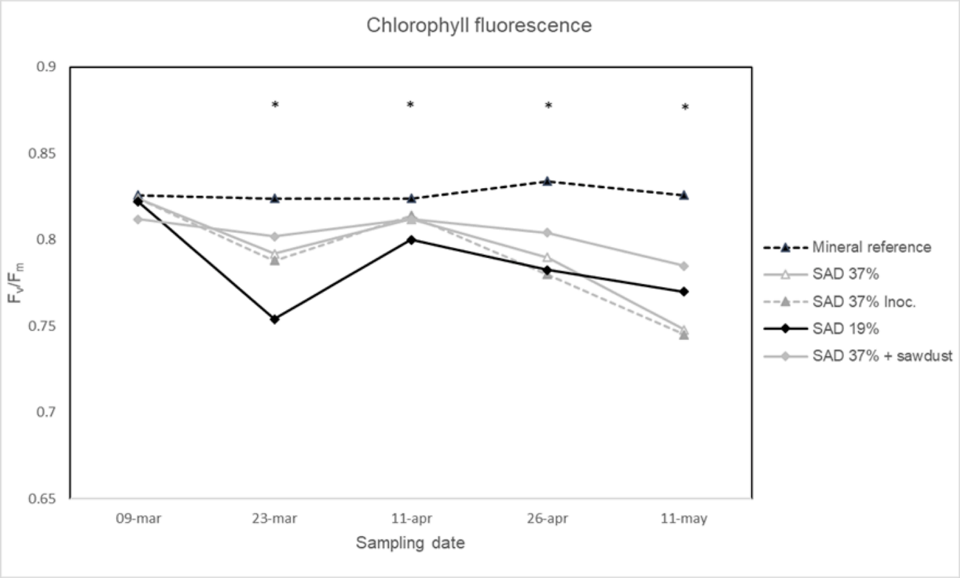

Fig. 2. Chlorophyll fluorescence value (F_v/F_m) in tomato plants grown in four different peat-based substrate mixtures with solid anaerobic digestate (SAD, 19 % or 37 % v/v), or peat substrate fed with mineral nutrient solution (reference), measured at five occasions during the experiment. $n = 5$. * denotes significant ($p<0.05$) differences between treatments within sampling occasion.

and Ågren, 1995). In a recent review, (Bergstrand, 2022) compiled the general relative requirement of nutrients in fertilizer and found that, with the N concentration in fertilizer set to 100, the relative requirement of the other macronutrient is: P 13–19, K 45–80, S 8–9, Mg 5–15, and Ca 5–15. On setting the available N value in this study (Table 3) to 100, the levels of other macronutrients, except S, were high compared with the general requirement cited above. This nutrient imbalance might be one reason for the lower growth compared with the mineral reference even though the total concentration of nutrients was high. The relative abundance of micronutrients was within the acceptable limit except for Zn and Cu, levels of which were rather high.

Uptake efficiency of the macronutrients from substrates including SAD was highest in the treatment with 19 % SAD (Table 6), but the total amount of nutrients taken up was still not enough to give the same growth as in treatments with twice as much added nutrients (37 % SAD treatments). In the treatment with 37 % SAD and part of the peat replaced with sawdust, uptake efficiency and DW were both lower. This can be explained by the approximately 54 % lower availability of NH_4 than in the treatment with 37 % SAD and no sawdust. The C/N ratio when sawdust was added was 40, while the 37 % SAD treatment had a C/N ratio of 30. According to White (2005), at C/N ratio >30 % NH_4 is fixed by microorganisms and temporarily redrawn from the available N pool, which may explain the lower nutrient utilization in the sawdust treatment. However, the availability of several other essential nutrients in addition to N was also decreased in the sawdust treatment, pointing at a fixation of other elements and perhaps fixation to binding sites on the sawdust itself and not only to microorganisms. Cation exchange capacity in reported to increase over time in substrate that includes sawdust (Lax et al., 1986), which may explain the decreased availability of several nutrients in the 37 % SAD-sawdust treatment. Another explanation for the reduced growth, and thus lower nutrient uptake, could be phytotoxicity effects of fresh forestry by-products, such as sawdust, which can affect growth of e.g., tomatoes (Dorais et al., 2007) in the case of certain tree species, while others do not cause any toxicity. Concentrations and production of toxic substances such as heavy metals, terpenes, and phenols are likely to be responsible for the toxicity symptoms.

Chlorophyll fluorescence parameter F_v/F_m is often used as an indicator of the general stress level of plants, with an average value of 0.83 considered normal for unstressed vascular plants (Björkman and Demmig, 1987). In this study, plants in all treatments had F_v/F_m values close to 0.83 one month after planting. However, at later stages all the plants growing in substrate with SAD had lower F_v/F_m values, while the mineral reference had values close to the optimum throughout cultivation. At harvest, all SAD plants had values below 0.8, clearly indicating stress. The reason for the increased stress is unclear, but it may be due to the nutrient imbalance observed.

Inoculation of the growing medium with active nitrifying bacteria did not have a significant effect on N availability or uptake. Thus could be because conditions such as pH or moisture in the growing medium were not conducive to the nitrification process, or because the nitrifying bacteria present in the inoculant were not well adapted to the environment in the growing medium.

5. Conclusions

Use of SAD as a nutrient-rich constituent of growing medium for tomato plants resulted in lower production than in a mineral-fertilized reference treatment. The plants displayed indications of stress, such as reduced F_v/F_m readings, but nutrient content in the growing medium did not fully explain the inferior performance of plants fertilized with SAD. Further studies are required to optimize the use of anaerobic digestate in long-season greenhouse crops. Adding sawdust or inoculation of substrate with active nitrifying bacteria did not reduce the ammonium concentration in the growing medium in this study. An alternative may be to increase the C/N ratio by adding composted bark from tree species that do not cause phytotoxicity, or by adding other organic residues, such as straw. The ammonium concentration could also be lowered by inoculation with nitrification bacteria prior to planting in a set-up that provides oxygen for the aerobic nitrification process. It is important to reduce peat use in horticultural production and SAD remains a promising candidate, since it contains high amounts of plant-available plant and possesses physical characteristics suitable for pot production.

CRediT authorship contribution statement

Håkan Asp: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Karl-Johan Bergstrand:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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