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## VIRUSES DEMONSTRATE SELECTIVE SURVIVAL DURING SIMULATED ANAEROBIC DIGESTION OF PLANT BIOMASS

**Objective.** This research aimed at laboratory simulation of anaerobic digestion of plant biomass contaminated by a mixture of viruses with a wide host range to evaluate if viruses can retain their infectivity and pose a biohazard during the use of plant-based reusable resources such as digestate. **Methods.** Anaerobic digestion was simulated in air-tight desiccators using anaerobic bags and indicator strips to ensure proper conditions, and using fresh biomass of naturally-virus-infected plants. In one of the variants, a commercial mixture of anaerobic bacteria and enzymes was also added to see if it would be more efficient in terms of plant digestion and virus inactivation. For serological detection of the tomato mosaic virus, cucumber mosaic virus, and potato virus Y, DAS-ELISA was used with commercial diagnostic kits and positive controls. Absorbance values were measured at 405 nm. Total RNA was extracted using a RNeasy Plant Mini kit following the manufacturer's instructions. Molecular detection of viruses was carried out by two-step RT-PCR using specific oligonucleotide primers available from the literature. To check if the viruses can retain their infectivity after the anaerobic digestion of plant biomass waste, a bioassay was used where *Nicotiana rustica* plants producing necrotic lesions upon infection with many viruses were mechanically inoculated at the stage of 2 true leaves. For statistical processing of data, the mean and standard error were calculated using Microsoft Excel software. **Results.** After prolonged anaerobic digestion, tomato mosaic virus was still detected in plant residues, but it is important to note that it was also found in high concentration in the liquid phase. Hence, during the anaerobic digestion, a significant proportion of the virus was successfully eluted from plant residues into the digestate. Interestingly and contrary to tomato mosaic virus, cucumber mosaic virus has not practically eluted to the liquid phase. On the other hand, potato virus Y was supposedly completely decomposed during the anaerobic digestion of plant biomass waste, as it was not detected either by ELISA or by PCR.

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*It is likely that not all of the investigated viruses represent a potential biohazard in the context of the use of renewable resources. RT-PCR outcomes showed the presence of tomato mosaic virus and cucumber mosaic virus both in the solid and the liquid phases, augmenting the ELISA data. Lastly, bioassay results have confirmed that at least tomato mosaic tobamovirus may escape the aggressive process of waste decomposition and remain able to infect host plants raising issues about the safety of reusable plant resources. **Conclusions.** The survival of plant viruses during plant biomass waste processing was studied in the context of biological safety. The preservation of serological markers and genetic signatures of model viruses in the process of anaerobic digestion has been confirmed. For the first time, it was proven that tomato mosaic virus may preserve its infectivity during long-term incubation of plant biomass waste in anaerobic conditions, which requires further attention when using renewable resources. On the example of PVY, it has been shown that potyviruses do not survive during anaerobic digestion and therefore may not pose an epidemic risk in using processed renewable resources. It has been demonstrated that artificial introduction of anaerobic microorganisms does not have a decisive effect on the stability of the studied model viruses.*

**Keywords:** viruses, survival, infectivity, plant biomass, waste treatment, anaerobic digestion.

Modern bioeconomy involves the reuse of many resources (primarily, wastewater and plant biomass waste), the implementation of the closed cycle of production (including organic farming) and its general environmentalization. Recycle and reuse of waste resources nowadays become more and more important and even required in many countries.

On June 26, 2023, the European Union introduced new regulations for the safe reuse of treated wastewater to preserve natural water resources ([https://environment.ec.europa.eu/topics/water/water-reuse\\_en](https://environment.ec.europa.eu/topics/water/water-reuse_en)). Similar trends are observed in the use of plant biomass waste which is now seen as a powerful and sustainable source of renewable energy in terms of biogas, biofuel, and combustible materials (pellets, etc.) [1, 2]. Major part of plant biomass waste is nowadays processed mainly by composting or anaerobic digestion (AD) [3, 4] with the formation of compost or mulch, or digestate with a high content of ammonia and phosphorus, which is subsequently used for soil protection and fertilization [5], especially in organic production [6].

Viruses are a major threat that is currently uncontrolled and requires the development of effective risk management approaches, especially in the light of crop yield declines, climate change, and reuse of bioresources (in particular, plant biomass waste). The latest studies indicate the emergence of new and the spread of modi-

fied viruses of strategically important plant crops in Ukraine, which ultimately leads to their accumulation in ecosystems [7-11].

It remains largely unknown if viable viruses can be actually present in such effluents from digesters or compost, which raises questions about the safety of their use and needs for a redesign of technological processes. However, there is an increasing body of evidence suggesting that many plant viruses can possibly survive the biomass treatment processes which currently mostly rely on composting or AD.

Many plant viruses are known for their high or even extreme stability in the environment. This is especially true for such widespread and «undemanding» viruses as tobamoviruses (tobacco mosaic virus (TMV), pepper mild mottle virus (PMMoV), tomato mosaic virus (ToMV)), cucumoviruses (cucumber mosaic virus (CMV)), etc. Ultimately, such robust viruses may be able to survive in plant debris (remains of previously infected plants after treatment), soil, humus, water, etc. and even on hard surfaces including laboratory or garden benches, equipment, tools, and so on [12, 13].

In previous studies into the virome of AD systems by high-throughput sequencing, the nucleotide sequences of a few exogenous plant viruses have been detected, which belonged to the *Virgaviridae*, *Betaflexiviridae*, *Tombusviridae*, *Potyviridae*, and *Secoviridae* families [14]. It

indicated potential existence of these viruses in the system as were initially introduced with the feedstocks [15], such as cucumber green mottle mosaic virus, tobacco mosaic virus, etc.

The infection and transmission potentials of such viruses in waste treatment processes still remain unclear. Hence, plant viruses may pose a real risk of inducing infectious diseases during further reuse of such renewable resources, including the use of waste residuals as soil fertilizers and recycled water for irrigation. This underpins the importance to evaluate the biosafety of using biomass waste-originated products like digestate or compost.

In addition to purely biological issues at hand, the destruction of infrastructural phytosanitary facilities due to military actions and the appearance of new plant viruses in Ukraine acutely raise the issue of biological safety of exploiting reusable resources that can cause disease outbreaks.

This paper describes the results of small-scale laboratory simulation of anaerobic digestion of plant biomass simultaneously contaminated with several common plant viruses to see if these pathogens can retain their biological properties (i.e., infectivity). By using a set of complementary techniques, we show that at least one of model viruses has successfully survived this simulation and was able to induce productive and symptomatic infection in susceptible host plants.

**Materials and Methods.** The laboratory implementation of AD technology and the general arrangement of small non-flow fermenters were based on the recommendations given in the work of Li et al. [16]. To simulate AD conditions in the laboratory, desiccators with a volume of 3 liters with a polished lid were used. To ensure the air tightness, the contact surface was coated with petroleum jelly. To ensure anaerobic growing conditions, anaerobic bags AnaeroGen 3.5L (Thermo Scientific, Oxoid, Great Britain) were used (2 bags/desiccator). Microbiology Anaerotest (Sigma-Aldrich, Germany) indicator strips confirmed AD conditions.

Plant material (aerial parts of young tomato, tobacco, and potato plants) was used as the starting material for AD, which previously had been sampled in the field and confirmed by ELISA/PCR methods as infected with ToMV, CMV, and potato virus Y (PVY). The material was weighed, chopped with sterile scissors, and loosely spread in an even mass at the desiccator bottom. Subsequently, sterile distilled water (1:1, m/v) was added to the plant material in the case of control. In the case of the AD experiment, sterile distilled water was amended with the commercial mixture of anaerobic microorganisms and enzymes (70% water + 30% mixture of microorganisms to general ratio with plant material 1:1, m/v), which is widely marketed in Ukraine. Airtight desiccators with virus-containing plant material were then incubated in a thermostat at 28 °C for ~2 months with a one-time addition of water (75 mL, control) or water with the commercial mixture of anaerobic microorganisms and enzymes (60 mL + 15 mL, AD experiment) 3 weeks after the start of fermentation with appropriate replacement of the anaerobic bags and indicator strips.

Digested plant material was divided into 2 phases: a solid phase (partially digested plant residues, SP) and a liquid phase (the digestate itself, i.e. the water that was added at the beginning of the experiment and the digestion products of plant material dissolved in the aqueous phase, LP). SP Samples were homogenized in 0.1M phosphate-buffered saline (pH 7.4) in a ratio of 1:2 (m/v) and centrifuged at 5000 rpm at 4 °C for 20 min to remove the debris. The supernatant was used for further detection of virus antigens. LP was purified by centrifugation as described above. SP and LP supernatant samples was then used for virus detection via the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), reverse transcription polymerase chain reaction (RT-PCR), and bioassay. The initial plant material ( used for digestion) served as a positive control in ELISA.

Samples of SP and LP were tested for ToMV, CMV and PVY by DAS-ELISA, as described by Clark and Adams [17], using specific diagnostic kits and positive controls from Loewe Biochemica GmbH (Sauerlach, Germany) and following the manufacturer's recommendations. Sap from healthy plants was used as negative controls. Absorbance values at 405 nm were measured using a BioTek Quant Microplate Spectrophotometer (Bio-Rad, USA) with KC Junior software. The absorbance values measured 60 min after adding the substrate, more than three times those of the negative controls, were considered positive.

Total RNA was extracted from SP and LP samples using a RNeasy Plant Mini kit (Qiagen, Germantown, MD, USA) and following the manufacturer's instructions.

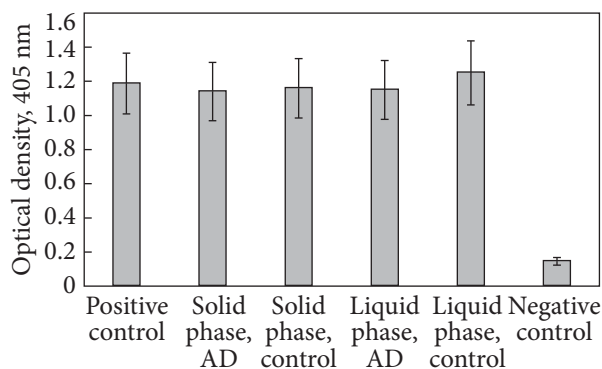
Two-step RT-PCR was performed using a Genetic Research Instrumentation Ltd thermocycler (Braintree, United Kingdom). The following oligonucleotide primers for amplifying a part of the coat protein gene of the respective virus were used: CMV-F (TATGATAAGAAGCTT-GTTTCGCGCA) and CMV-R (TTTTAGC-CGTAAGCTGGATGGACAACCC) for CMV detection producing a fragment of 500 bp [18], and forward primer (CGGAAGGCCTAAAC-CAAAAAG) and Tob-Uni1 primer (ATTTA-AGTGGAGGGAAAAACACT) for ToMV detection amplifying a fragment of 686 bp [19]. For the reverse transcription, RevertAid Reverse Transcriptase (MMuLV RT; Thermo Scientific, USA) was used. The reverse transcription mixture contained 4 µL of 5X reaction buffer (Thermo Scientific, USA), 2 µL of dNTP at a final concentration of 1 mM, and 1 µL reverse transcriptase (200 U). The mixture was incubated at 42 °C for 1 hour. Amplification was carried out in 12.5 µL of Dream Taq PCR Master Mix (2x) buffer (containing Dream Taq DNA polymerase, 2X Dream Taq buffer, 0.4 mM of each dNTP and 4 mM MgCl<sub>2</sub>) (Thermo Scientific, USA), 7.5 µL of sterile water, 1 µL of each primer (10 µM), and 3 µL of cDNA obtained at the RT step. The am-

plification reactions were set up as follows: initial denaturation for 3 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50–60 °C (depending on the virus) for 30 s, and extension at 72 °C for 45 s. The final extension was at 72 °C for 10 min. The PCR products with Thermo Scientific MassRuler DNA Ladder Mix (Thermo Fisher Scientific, Waltham, MA, USA) were separated in a 1.5% agarose gel stained with ethidium bromide and visualized under UV light [20].

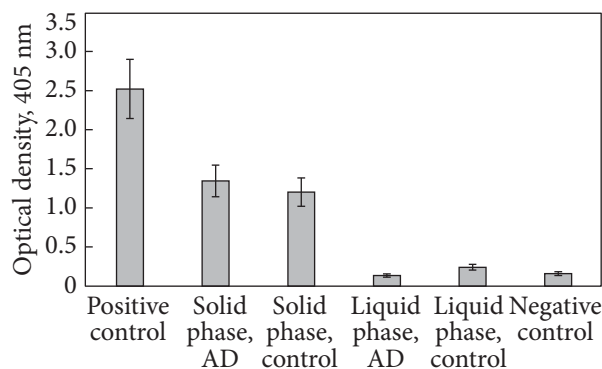
To check if the viruses can retain their infectivity, i.e., survive, after the AD of plant biomass waste, *Nicotiana rustica* plants producing necrotic lesions upon infection with many viruses, including ToMV, were mechanically inoculated using carborundum powder at the stage of two true leaves. Plants were inoculated with filtered SP and LP samples after the AD (experimental group) with filtered sap of infected plants (positive control group) or with sap of healthy plants (negative control group). Each group contained at least 5 plants.

All biological experiments were repeated at least 3 times. In addition to biological replicates, experimental replicates were observed using appropriate internal and external positive and negative controls (where applicable — ELISA and RT-PCR). For statistical processing of data, the averages and standard errors were calculated using Microsoft Excel software.

**Results.** For the AD, we used vegetative mass of tomato, tobacco, and potato plants previously confirmed as infected with ToMV, CMV, and PVY. To evaluate the importance of artificially introduced bacteria as stimulators of AD, the biomass in one of the desiccators was amended with the commercial mixture of anaerobic microorganisms and enzymes diluted in sterile distilled water. Apart from this, all the remaining conditions in two desiccators (biomass content and weight, total volume of diluent added, total volume of the desiccator, duration and temperature of incubation) were identical. Anaerostest



**Fig.1.** Detection of serological markers of tomato mosaic virus (ToMV) in plant samples and digestate



**Fig.2.** Detection of serological markers of cucumber mosaic virus (CMV) in plant samples and digestate

indicator strips confirmed strict anaerobic conditions in both desiccators.

After the AD, visual differences between the plant biomass from two desiccators were noted: the biomass became softer and more yellowish when the commercial mixture of microorganisms and enzymes was added as compared to plant material digested without artificially added bacteria, which may be indicative of more efficient decomposition of plant waste. The resulting volume of the digestate, however, was similar in both desiccators.

For serological detection of model viruses introduced into the digestion system in both plant

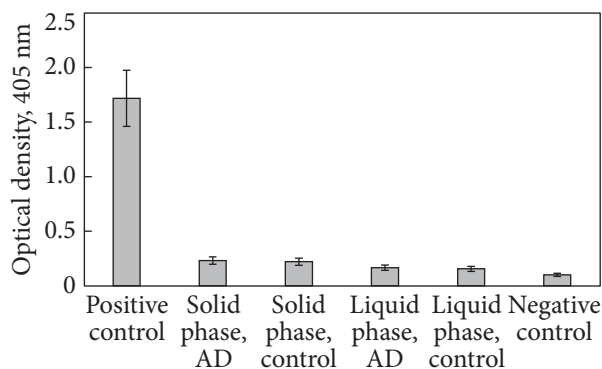
samples and digestate, a proven DAS-ELISA technique was used. As described above, the supernatant samples for ELISA were derived from the SP and LP phases. Detailed description of sample groups used for detection of ToMV, CMV, and PVY is given in Table 1.

As seen in Fig. 1, ToMV was present in the initial plant material (positive control) in high concentration. After prolonged anaerobic digestion, it was still detected in plant residues (solid phase, both AD and control) in high concentration. Finally, it is important to note that this virus was also found in high concentration in the liquid phase. In other words, during the AD, a signifi-

**Table 1. Sample groups of plant material and digestate used for serological detection of viruses**

Sample group	Description
Positive control	Original plant material (a mixture of plants naturally infected with ToMV, CMV, and PVY) later used for AD
Solid phase, AD	Solid plant remains after AD with the addition of commercial mixture of anaerobic microorganisms and enzymes
Solid phase, control	Solid plant remains after AD without adding commercial mixture of anaerobic microorganisms and enzymes
Liquid phase, AD	Digestate (liquid products of AD with the addition of commercial mixture of anaerobic microorganisms and enzymes)
Liquid phase, control	Digestate (liquid products of AD without adding commercial mixture of anaerobic microorganisms and enzymes)
Negative control	Partially purified sap of healthy plant

The samples described in Table 1 were then used for detection of three viruses: ToMV, CMV, and PVY (Figs. 1-3).



**Fig. 3.** Detection of serological markers of potato virus Y (PVY) in plant samples and digestate

cant proportion of the virus was successfully eluted from plant residues into the digestate.

According to Fig. 2, the initial plant biomass also contained high level of CMV. However, after long-term anaerobic incubation, CMV content halved in the SP, regardless of the presence of artificially added anaerobic microorganisms during the AD. Interestingly and contrary to ToMV, CMV was not practically eluted to the LP, regardless of the presence of anaerobic microorganisms during the AD. The content of viral antigens in the LP was roughly identical to the negative control.

Fig. 3 represents the PVY detection in the same groups of samples. As shown, like the two previous viruses, PVY was also present in the initial biomass (positive control) in high concentration. However, in contrast to both of the viruses, incubation of PVY-containing plant material under anaerobic conditions, regardless of the presence of artificially introduced anaerobic microorganisms during the AD, likely resulted in virtually complete inactivation of the virus, as viral antigens were not reliably detected by ELISA either in SP or in LP (Fig. 3): the virus content was statistically similar to that in the sap of healthy plant (negative control).

Summarizing the results of serological detection of three model plant viruses in digested plant biomass and digestate after the AD, it is

worth noting that these pathogens demonstrated different stability to the digestion and contradictory content in solid and liquid phases. It is likely that not all of the investigated viruses represent a potential biohazard in the context of the use of renewable resources.

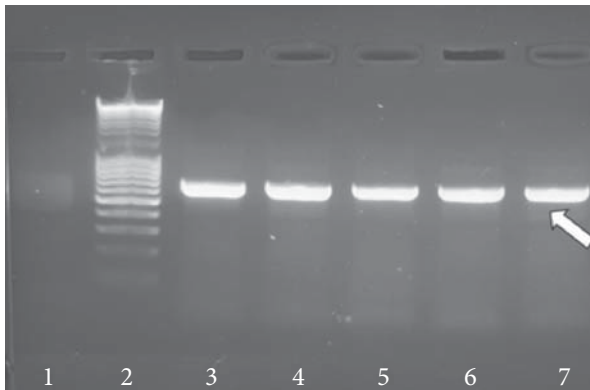
Further, it was necessary to confirm the obtained data by an independent method of molecular diagnostics using the polymerase chain reaction. Such double confirmation of the viral nature at the protein and gene levels is a common practice for conducting similar studies.

Based on the results of serological detection of plant viruses after the AD given above, only ToMV and CMV were detected in the experimental samples. Hence, RT-PCR was performed only for these two viruses preserved in the SP and/or LP, but not for PVY (Figs. 4, 5). For ease of perception, the sample groups shown in Figs. 4, 5 are identical to those given in Table 1 and Figs. 1-3.

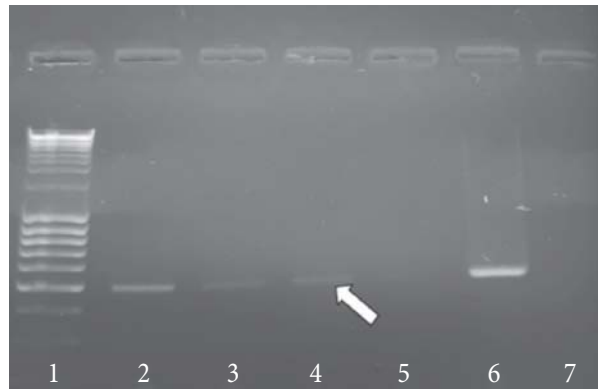
Fig. 4 shows that ToMV was detected in the solid and liquid phases (tracks 3-6) regardless of the presence of artificially introduced anaerobic microorganisms, which is consistent with the ELISA results presented in Fig. 1. Similarly, ToMV was detected in the original plant material (positive control, track 7) but was not found in healthy plants (negative control, track 1).

RT-PCR for detection of CMV yielded similar results (Fig. 5). In particular, CMV was identified in SP (tracks 3-6) regardless of the presence of artificially introduced anaerobic microorganisms, which is consistent with the ELISA results presented in Fig. 2 and is similar to the results obtained for ToMV. However, in contrast to ELISA, PCR also reliably confirmed the presence of CMV genetic signatures in LP after the AD with artificially introduced anaerobic microorganisms (track 4), but not in the LP without them (track 5), which may be indicative of more effective elution of CMV from plant residues.

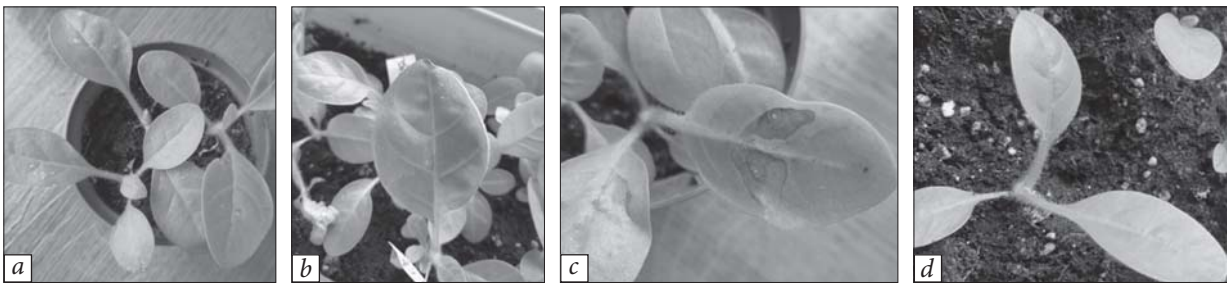
Notably, the results of molecular detection of CMV in different groups of samples predictably



**Fig.4.** Visualization of PCR products with primers specific to ToMV (expected size of the amplification product 686 bp): 1 — negative control; 2 — Thermo Scientific MassRuler DNA Ladder Mix; 3 — solid phase, AD; 4 — solid phase, control; 5 — liquid phase, AD; 6 — liquid phase, control; 7 — positive control. The target product is indicated by an arrow



**Fig.5.** Visualization of PCR products with primers specific to CMV (expected size of the amplification product 500 bp): 1 — Thermo Scientific MassRuler DNA Ladder Mix; 2 — solid phase, AD; 3 — solid phase, control; 4 — liquid phase, AD; 5 — liquid phase, control; 6 — positive control; 7 — negative control. The target product is indicated by an arrow



**Fig.6.** Local necrotic symptoms of virus infection on *N. rustica* plants inoculated with partially purified samples of (A) solid phase; (B) liquid phase; (C) positive control; and their absence in (D) negative control

were more accurate than the results of ELISA (Fig. 2) and, unlike the latter, confirmed the presence of genetic material of CMV in the LP.

Summarizing the outcomes of the molecular detection of two model plant viruses in digested plant biomass and digestate after the AD, it should be noted that the obtained data are consistent with the results of serological diagnostics and complement them by the detection of CMV in LP with artificially introduced anaerobic microorganisms.

The results of serological and molecular diagnostics of model plant viruses after the AD of virus-containing plant residues show that only

ToMV, one of the three investigated here viruses, poses a high potential threat to biosecurity. This virus was preserved in the SP of plant residues (with or without artificially introduced anaerobic microorganisms), then effectively eluted to the LP, highly stable in the environment. It is mechanically transmitted and has a wide range of host plants. Taking all this into account, bioassay (confirmation of the preservation of virus infectivity, i.e., its survival) was carried out exclusively for ToMV. For this, we used young healthy plants of *N. rustica*, which reacts to ToMV infection by developing local lesions (Fig. 6).

As can be seen from Fig. 6, plants reacted identically to inoculation with virus-containing material, regardless of which phase it was (either solid or liquid) and whether anaerobic microorganisms were artificially introduced to the desiccator in the case of the AD, which is in accordance with ELISA and PCR data.

Since the local necrotic reaction of plants allows for a quantitative assessment of the viral load, it can be stated that, in general, the solid and liquid phases of the digested plant material contain approximately the same number of infectious virus particles (multiple confluent necroses are visible) (Fig. 6A-D).

Summarizing the results of bioassay, it should be noted that the survival (preservation of infectious properties) of plant viruses after anaerobic digestion was confirmed for the first time for tomato mosaic tobamovirus (ToMV). This pathogen was detected by three complementary methods (ELISA, PCR and bioassay) and has successfully retained its biological properties in an aggressive environment.

**Discussion.** In this work, we have simulated the conditions of anaerobic digestion in the lab to see if plant viruses might still remain virulent after the decomposition of plant biomass. Three different viruses studied here, namely ToMV, CMV, and PVY, demonstrated varying elution to the resulting digestate and overall survival rate. During the incubation, a significant proportion of ToMV was successfully eluted from plant residues. This aspect is particularly noteworthy, since ToMV is a tobamovirus, which is known for its stability in the environment and, moreover, is transmitted mechanically [12-15, 21, 22]. Thus, effective elution of antigens of this virus to LP constitutes a potential biological hazard, since, provided that its infectious properties are preserved, the virus may eventually reach ground or surface water/fertilizers/environment during composting/processing of plant waste, where it could remain able to infect susceptible plants. This is in agreement with a recent study

of the virome of irrigation water which suggests a correlation between viruses present in water and those found in susceptible plants possibly leading to the outbreaks [23]. ToMV has a wide range of hosts and, therefore, in view of the above, has a high epidemic potential. Notably, artificial addition of anaerobic bacteria during the incubation of virus-containing plant material did not have a noticeable effect on the virus content in the SP, as well as on the efficiency of its elution to the liquid phase. In other words, it can be assumed that other methods of plant waste processing (except the AD) also may not ensure effective removal/destruction of tobamoviruses.

For CMV, however, we have shown that the content of viral antigens in the LP was at the level of the negative control, that is, at the limit of ELISA sensitivity. Thus, the virus was still present in plant debris at a high enough concentration after the AD, but did not elute into the LP, potentially reducing its biohazard when re-using resources. The reasons for the ineffective elution of CMV to the LP are unclear and were not investigated here. In our opinion, they probably lie in the peculiarities of the morphology of this virus, since it is the only icosahedral virus among the described in this paper. Combined with the small size (~25 nm in diameter), the spherical shape of CMV virions is likely to favor their association with plant debris and hinder elution into the LP.

It is likely that not all of the investigated viruses represent a potential biohazard in the context of the (re)use of renewable resources. In particular, the incubation of plant residues led to almost complete removal/destruction of PVY, a harmful pathogen with a wide host range. It is known that potyviruses are not stable in the environment, and we suggest that they might be effectively inactivated during plant biomass waste processing. In contrast to PVY, ToMV and CMV persisted in high concentration in the SP of plant waste, and therefore its use as a fertilizer requires prior inactivation of the viruses by other means if available.



Finally, only ToMV was found in high concentration in the LP, which is in agreement with the previous data on tobamovirus stability and of particular concern since it is a liquid phase (digestate) that is considered promising for use as a fertilizer after the AD. In broader context of plant waste processing (for example, composting), the survival of ToMV and its effective elution to the LP means the possibility of the virus getting into sewage, soil, and rainwater and its further spread, as discussed above. Since tobamoviruses are very common and stable in the environment, are transmitted mechanically (without the involvement of vectors), have a wide host range, and lead to significant crop losses, it is tobamoviruses that are currently considered the most dangerous viral agents requiring attention when processing the plant biomass waste and (re)using renewable resources.

To our knowledge, this is the first biological proof of the plant virus retaining its infectivity after the anaerobic digestion of virus-contaminated plant biomass. Data resulting from our small-scale lab experiment indicate that at least some viruses may escape the aggressive process of waste decomposition and remain able to infect host plants raising issues about the safety of reusable plant resources. In the view of aforesaid, more research is required on using industrial digesters employed in agriculture for mass-scale digesting of plant biomass waste to determine

crucial technological steps where highly stable plant viruses could be inactivated. Also, practical input on tobamoviruses' stability and inactivation means is needed as these pathogens seem to be extremely effective in evading existing 'sterilizing' tools typical for digesters and used for water reclaim.

**Conclusions.** The survival characteristics of model plant viruses during biomass waste processing and resource reuse in the context of biological safety have been studied. The preservation of serological markers and genetic signatures of the model viruses ToMV and CMV in the process of anaerobic digestion has been confirmed. For the first time, it has been proven that model ToMV may preserve its infectivity during long-term incubation of plant biomass waste in anaerobic conditions, which requires further attention when using renewable resources. On the example of PVY, it has been shown that potyviruses do not survive during the anaerobic digestion and therefore may not pose an epidemic risk when using processed renewable resources. It has been demonstrated that artificial introduction of anaerobic microorganisms does not have a decisive effect on the stability of the studied model viruses.

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## ВІРУСИ ДЕМОНСТРУЮТЬ ВИБІРКОВЕ ВИЖИВАННЯ ПІД ЧАС СИМУЛЯЦІЇ АНАЕРОБНОГО ТРАВЛЕННЯ РОСЛИННОЇ БІОМАСИ

**Мета.** Дослідження було спрямоване на лабораторне моделювання анаеробної ферментації рослинної біомаси, контамінованої сумішшю вірусів рослин, що мають широкий спектр рослин-хазяїв, для оцінки того, чи можуть віруси зберігати свою інфекційність і становити біологічну небезпеку під час використання рослинних ресурсів багаторазового використання, таких як дигестат. **Методи.** Анаеробна ферментація була змодельована в герметичних ексікаторах з використанням анаеробних пакетів та індикаторних смужок для забезпечення належних умов, а також з використанням свіжої біомаси рослин, природно заражених вірусами. Один із дослідних варіантів містив комерційну суміш анаеробних бактерій і ферментів для підсилення ферментації рослин. Детекцію вірусів здійснювали за допомогою DAS-ELISA з комерційними діагностичними наборами та позитивними контролями. Значення абсорбції одержували при довжині хвилі 405 нм. Тотальну РНК виділяли за допомогою набору RNeasy Plant Mini kit, дотримуючись інструкцій виробника. Молекулярну детекцію вірусів проводили за допомогою двоетапної RT-PCR з використанням специфічних олігонуклеотидних праймерів. Вивчення збереження інфекційності вірусів після анаеробної ферментації відходів рослинної біомаси проводили за допомогою біотестування на рослинах *Nicotiana rustica*. **Результати.** Після тривалої анаеробної ферментації вірус мозаїки томату детектувався у рослинних рештках. При цьому важливо відзначити, що цей вірус також був виявлений у високій концентрації в рідкій фазі. Отже, під час анаеробної ферментації значна частина вірусу успішно елюювала з рослинних решток до дигестату. Слід зазначити, що вірус огіркової мозаїки, на відміну від вірусу мозаїки томату, практично не елюював до рідкої фази. З іншого боку, Y-вірус картоплі був повністю деградований під час анаеробної ферментації відходів рослинної біомаси, оскільки його не можна було детектувати ані методом ІФА, ані ПЛР. Цілком імовірно, що не всі досліджені віруси становлять потенційну біологічну небезпеку в контексті використання відновлюваних ресурсів. Результати RT-PCR показали наявність вірусу мозаїки томату та вірусу огіркової мозаїки як у твердій, так і в рідкій фазах, що доповнює дані ELISA. Нарешті, результати біотестування підтвердили, що принаймні тобамовірус мозаїки томату може уникати агресивного процесу розкладання відходів і залишатися здатним інфікувати рослини-хазяї, що викликає питання щодо безпеки рослинних ресурсів багаторазового використання. **Висновки.** Вивчено характеристики виживання вірусів різних родин під час переробки відходів біомаси у контексті біологічної безпеки. Підтверджено збереження серологічних маркерів та генетичних сигнатур модельних вірусів у процесі анаеробної ферментації. Вперше доведено, що вірус мозаїки томату може зберігати свою інфекційність впродовж довготривалої інкубації відходів рослинної біомаси в анаеробних умовах, що потребує додаткової уваги при використанні відновлювальних ресурсів. На прикладі Y-вірусу картоплі доведено, що потівіруси не виживають під час анаеробної ферментації і тому можуть не становити епідемічного ризику при використанні відновлювальних рослинних ресурсів. Показано, що штучне додавання анаеробних мікроорганізмів не має вирішального впливу на стабільність досліджуваних вірусів.

**Ключові слова:** віруси, виживання, інфекційність, рослинна біомаса, переробка відходів, анаеробна ферментація.