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VIRUS OCCURRENCE AND SURVIVAL IN REUSABLE RESOURCES: A MINIREVIEW

This work covers important aspects of the occurrence and viability of various viruses in the two most common reusable waste resources: wastewater and biomass waste. Detection of human, bacterial and plant viruses in these wastes are summarized. Historically, human viruses have been monitored in wastewater for decades. Evidence suggests that wastewater mostly contains fecal-orally transmitted viruses, which are abundant and diverse. Recently, an increasing occurrence of SARS-CoV2 in sewage water with the spreading epidemics has been confirmed but lacking biological proof of infectivity yet. Besides human pathogens, wastewater is shown to be rich in bacteriophages and plant viruses as well, which supposedly enter the water from human guts. Viruses serving as water quality indicators are also discussed here. Lastly, we focus on biomass waste treatment, showing the presence of some common and stable plant viruses which may supposedly survive the technological process.

Keywords: *viruses, bacteriophages, survival, waste treatment, anaerobic digestion, metagenomics*

Nowadays the recycle and reuse of waste resources become more and more important and even requirements in many countries. The long-standing leader here is wastewater. Wastewater is treated to remove contaminants both abiotic (particles, organic matter, N, P, etc.) and biotic (bacteria, protozoa, fungi, toxins, viruses, etc.). Further, treated water is often reused for different technological processes (washing, cooling, heating, etc.), irrigation of glasshouses or open fields, and in livestock farming as well.

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By applying a set of techniques, wastewater treatment aims at maximum purification. The reality, however, is that many organisms may eventually escape this process, leading to high titers of *Escherichia coli* and other bacteria in some areas. The same is true for viruses. The occurrence of viruses in renewable resources is of utmost importance. Since such resources are suggested for further reuse, pathogens existing therein may potentially induce diseases in humans, animals, or even plants if proven viable. This risk was demonstrated for many human enteric viruses as reviewed below.

Another major side of the story is biomass waste treatment. Biomass wastes, including food waste, agriculture waste, and activated sludge, are generally treated with biotechnologies like anaerobic digestion (AD) to achieve both waste reducing and resource recycling. AD has been widely used for waste and wastewater treatment with the recovery of renewable energy in the form of CH_4. Digestate, the residual effluent from anaerobic digesters, is high in ammonia and phosphorus and can be used as organic fertilizers for plants. It remains largely unknown if viable viruses can be present in such effluents, which raises questions about the safety of their use and the need for a redesign of technological processes.

This paper provides a brief overview of available data about different viruses present in renewable resources and the risks they impose or may impose for biosafety.

Human viruses in wastewater. It has been recently recognized that the treatment of used or waste water does not provide efficient purification from human viruses [1]. Despite many different procedures being used to remove physical, chemical, and microbiological wastes from sewage, high water purification efficiency does not demonstrate adequate reduction of virus levels. Additionally, in many regions of the world, especially in developing countries, the respective standards for water treatment are either missing, not followed properly, or do not include virus reduction requirements (Ukraine here is a good example).

Several types of human viruses are known to be present in sewage. Most commonly, these are enteric viruses transmitted via the fecal-oral route, which are released in huge numbers by infected individuals into the sewage system, causing so-called waterborne illnesses. Popular types include noroviruses, sapoviruses, rotaviruses, astroviruses, picornaviruses, and adenoviruses [2–5]. Most of the human enteric viruses are quite stable in adverse conditions, such as wide ranges of pH or temperature, and may even be resistant to chlorination [6, 7], making them difficult to remove from water by normal treatments. In addition, there were studies describing polyomaviruses and human coronaviruses (SARS-CoV2) as important viral pathogens present in sewage and treated water in large quantities [8–11]. Back in 2020, the Center for Disease Control launched the National Wastewater Surveillance System in response to the COVID-19 pandemic [12]. A quantitative polymerase chain reaction is used to analyze SARS-CoV2. With this method, only the virus RNA level (i.e., the number of virus genome copies) is measured, not the virus itself.

It is worth noting that for some viruses, we still lack 'a proof of life', as these pathogens have only been identified molecularly and/or serologically (a rarer case), but their infectivity is still questioned. SARS-CoV2 is the biggest challenge here (nevertheless, there are no proven cases of people infected due to exposure to wastewater). This is however not the case for 'true' enteric viruses causing waterborne epidemics for years. Luckily and in contrast to bacteria, the counts of pathogenic viruses in sewage are considered relatively small, in which only one single virus particle may exist among a million coliforms [4, 13].

Viruses of bacteria and plants in wastewater. Apart from viruses, bacteria are the most numerous life forms on the Earth and hence are very abundant in every environment. Many wastewater treatment processes remove bacteria, but no process can remove all microorganisms from the treated water. Water, especially contaminated wastewater, is extremely rich in microorganisms. Their amount can reach 10^5-10^9 bacteria/ml of sewage. In many regions, *E. coli, Clostridium* sp., *Legionella* sp., *Mycobacterium* sp., *Shigella* sp., *Vibrio* sp., *Salmonella* sp., Shigella sp., *Streptococcus* sp., and *Pseudomonas* sp. may be common among the other less numerous bacteria in sewage [14, 15], and most of them are either opportunistic or pathogenic. Moreover, these pathogenic bacteria may even form seasonal communities in wastewater [16].

Logically, the diversity and abundance of bacterial members in wastewater indicate that inevitably there can be even more bacterial viruses (or bacteriophages). Phages probably form the dominant group of viruses in sewage or any other type of used water. This could also be due to the fact that the human gut virome that is excreted into the sewage with feces is dominated by bacteriophages [17]. Since *E. coli* is thought to be the most numerous bacteria in wastewater, the majority of bacterial viruses discovered so far in the sewage were diverse RNA and DNA coliphages [18]. The actual phage diversity, however, should be much higher and depend on the availability of hosts. As discussed above, apart from *E. coli* viruses, phages of *Pseudomonas* sp., *Streptococcus* sp., *Shigella* sp., etc. should be also expected in wastewater.

Metagenomic studies indicated the occurrence of a highly abundant bacteriophage called crAssphage with a predicted *Bacteroides* host [19]. Later on, the human gut symbiont *Bacteroides intestinalis* was proven to be the actual host for this crAssphage [20], verifying the previous *in silico* predictions. The virus was a DNA phage in the order *Caudovirales* [21].

The occurrence of plant viruses in wastewater is more enigmatic, though. The initial question was how they enter the water. Presumably, our feeding behavior is the major source of plant viruses in the sewage. Some extremely stable viruses may eventually survive the hostile environments of human (and animals') guts. The virome analysis of gastrointestinal samples of children during the first year of life indicated colonization of fifteen different tobamovirus species. Among them, tropical soda apple mosaic virus, pepper mild mottle virus, tomato mosaic virus, and opuntia tobamovirus 2 were the most common representatives [22]. Also, it was demonstrated that pepper mild mottle virus (PMMoV) was a very stable tobamovirus that can survive during the transit through the human digestive tract and then be transmitted via water (also possibly including rainwater) with retaining its infectivity [23]. There were reports of tobamoviruses found in environmental waters and treated wastewater [24, 25], and even in drinking water [26]. These pathogens still remained infectious and survived in the nutrient solution for up to 0.5 years [27], suggesting an important role of water in the epidemiology of plant virus infections and transmission of these viruses. Further, another confirmation came showing water-mediated transmission of cucumber green mottle mosaic virus [28], which becomes increasingly relevant for hydroponically cultivated crops (i.e., mostly in glasshouses) [29]. Similar observations were also noted for tobacco mosaic virus (TMV), another iconic tobamovirus found in irrigation water [30].

Even though tobamoviruses are regarded as the dominant plant viruses in wastewater, the list of detected potentially pathogenic plant viruses is much longer, including the virus types threatening agricultural incomes, such as brome mosaic virus, cucumber mosaic virus (CMV), potato virus Y, and pepino mosaic virus [25, 31]. So far, four widespread groups of viruses have been recognized as waterborne pathogens endangering plant cultivation via irrigation: tobamo-, bromo-, cucumo-, poty-, and potexviruses. Worthy to note that the representatives of at least three of these groups typically have wide host ranges.

Viruses of bacteria and plants as indicators of pathogen contamination in wastewater. High titers of bacterial and plant viruses in wastewater (in parallel to pathogenic human viruses) raised questions on the possibility of their use to monitor the efficiency of water treatment technologies in terms of overall virus removal. By the theory, lower counts of phages or plant viruses (which are easier and safer to measure) should correlate with the reduced levels of human pathogenic viruses after treatment of contaminated water.

There are numerous works indicating the successful implementation of this idea into practice. For instance, recent data indicate that the removal rates of phages are similar to those of human viruses, and hence phages (in particular somatic coliphages) may represent more reliable indicators of water quality [32]. Also, the MS2 phage was demonstrated as a suitable monitoring and validating indicator in a multiple-barrier concept for wastewater treatment [33]. Lastly, the aforementioned crAssphage was demonstrated to be an effective tracking marker of water pollution [34–36].

In one of the first studies to evaluate the applicability of plant viruses conducted by Hamza et al. [37], all the wastewater samples tested were positive for PMMoV, contrary to several human viruses tested. Additionally, the high concentration and spread of PMMoV in sewage and river water suggest that this pathogen may be considered an efficient indicator of fecal pollution (or, vice versa, purification efficiency) for treated water.

Also, PMMoV was shown to be the most prevalent virus in both influent and effluent wastewater without notable seasonal changes in its level, showing an insignificant reduction during the treatment processes [38–40].

Later on, human pathogenic viruses including adenoviruses, noroviruses, rotaviruses, enteroviruses, polyomaviruses, etc. demonstrated relatively low concentrations detected and low correlations with water treatment efficiency. On the other side, PMMoV and TMV (both are tobamoviruses) were efficient indicators for predicting the pathogen contamination level in environmental water samples [35, 41].

However, this pattern may also depend on people's diet habitats in different areas, so other environmentally stable viruses may also be considered.

Plant viruses in biomass wastes. There is an increasing body of evidence suggesting that many plant viruses can survive the biomass treatment processes which currently mostly rely on composting and anaerobic digestion.

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 (TMV, PMMoV, tomato mosaic virus, etc.), cucumoviruses (CMV), etc., which can be mechanically transmitted from plant to plant without vectors. This feature adds to the feasibility of some viruses for efficient water transmission and survival, as mentioned above. 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 [42, 43].

When biomass waste from agriculture (i.e., plants, fruits, twigs, grass, etc.) is shredded and processed, viruses may supposedly survive. The subsequent use of resulting digestate or compost as fertilizers may endanger susceptible crops. Biosafety evaluation of the digestate product used as a soil organic fertilizer is however lacking.

In previous studies into the virome of AD systems by high-through-put sequencing, the nucleotide sequences of quite a few exogenous plant viruses have been detected, which belonged to the *Virgaviridae*, *Betaflexiviridae*, *Tombusviridae*, *Potyviridae,* and *Secoviridae* families [44]. This indicates the potential existence of these viruses in the system as being initially introduced with the feedstocks [45], e.g. a cucumber green mottle mosaic virus, tobacco mosaic virus, etc. Such tobamoviruses were also shown abundant and viable in wastewater.

The infection and transmission potentials of such viruses in waste treatment processes, however, still remain unclear. Hence, plant viruses may pose a real risk of inducing infectious diseases during further reuse of renewable resources, including the use of waste residuals as soil fertilizers and recycled water for irrigation, etc.

This underpins the importance to evaluate the biosafety of using biomass waste/wastewater-originated products like digestate or compost.

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ПОШИРЕННЯ ТА ВИЖИВАННЯ ВІРУСІВ У РЕСУРСАХ ПОВТОРНОГО ВИКОРИСТАННЯ: МІНІОГЛЯД

У цій роботі описано важливі аспекти появи та виживання різних вірусів у двох найбільш поширених ресурсах багаторазового використання: стічних водах та відходах біомаси. Узагальнено дані щодо виявлення вірусів людини, бактерій та рослин у цих відходах. Показано, що висока ефективність очищення води не завжди супроводжується відповідним зниженням вмісту вірусів. Зазвичай йдеться про ентеральні норовіруси, саповіруси, ротавіруси, астровіруси, пікорнавіруси та аденовіруси, що передаються фекально-оральним шляхом, які у величезних кількостях виділяються інфікованими особами в каналізацію. Крім того, описані поліома- та коронавіруси людини (SARS-CoV2) як важливі вірусні патогени, присутні у стічних водах та очищеній воді у великих кількостях. Однак, оскільки для аналізу SARS-CoV2 у стічних водах використовується кількісна полімеразна ланцюгова реакція, то інфекційність даного збудника і досі викликає сумніви. Вода, особливо забруднена стічна вода, надзвичайно багата мікроорганізмами. *E. coli*, *Clostridium* sp., *Legionella* sp., *Mycobacterium* sp., *Shigella* sp., *Vibrio* sp., *Salmonella* sp., *Shigella* sp., *Streptococcus* sp. і *Pseudomonas* sp. є найпоширенішими мікроорганізмами, здатними утворювати навіть сезонні угруповання. Велика кількість бактерій у стічних водах свідчить про високі титри бактеріофагів, які є домінуючою групою вірусів у стічних водах або будь-якому іншому виді використаної води. Крім того, було показано, що стічні води містять велику кількість рослинних вірусів. Ці патогени, ймовірно, можуть виживати в агресивному середовищі кишечника, що було підтверджено для деяких надзвичайно стабільних вірусів, а саме тобамо-, бромо-, кукумо-, поті- та потексвіруси, які також потрапляють в рослинні екосистеми через зрошення. Поява бактеріальних і рослинних вірусів у стічних водах, подібно до патогенних для людини вірусів, дозволила використовувати їх для контролю ефективності очищення води. Останні дані свідчать, що швидкість елімінації фагів зі стічних вод близька до такої для людських вірусів, і, отже, фаги можуть вважатися більш надійними та безпечними показниками якості води. Деякі віруси рослин (зокрема, вірус м'якої крапчастості перцю та деякі інші тобамовіруси) також запропоновано як ефективних індикаторів для прогнозування рівня контамінації патогенами людини проб води у довкіллі. Крім того, багато фітовірусів можуть вижити в процесах обробки біомаси шляхом компостування та анаеробного зброджування. Особливо це стосується таких поширених і стійких вірусів, як тобамо- та кукумовіруси, які можуть передаватися механічно. При переробці відходів біомаси в сільському господарстві віруси можуть виживати, а подальше використання отриманих добрив може поставити під загрозу сприйнятливі культури. У статті розглянуто віруси, що служать індикаторами якості та біобезпеки води і переробленої біомаси. Представлено інформацію щодо обробки відходів біомаси та наявності деяких поширених і стабільних вірусів рослин, які, ймовірно, можуть виживати в технологічному процесі.

Ключові слова: *віруси, бактеріофаги, виживання, переробка відходів, анаеробна ферментація, метагеноміка*