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A NOXIOUS WEED *AMBROSIA ARTEMISIIFOLIA L.* AS A SUSTAINABLE FEEDSTOCK FOR METHANE PRODUCTION AND METALS IMMOBILIZATION

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Noxious weeds are widespread in the countries of Europe, America, and Asia and pose a significant danger to the environment. The toxic metabolites of invasive plants have a greater impact on the plants and microorganisms that live in those biogeocenoses. Many of them, for example, the *Ambrosia* genus, also pose a danger to human health, causing allergic reactions as well as skin and respiratory tract irritation. In addition, invasive weeds can rapidly colonize agricultural and urban lands, inhibit the growth of valuable agricultural crops (allelopathic effect), and reduce the harvest. Thus, the problem of spreading these plants entails serious economic and social consequences. The plant *Ambrosia artemisiifolia* (family *Asteraceae*) is an especially noxious weed that can cause significant human health damage [1, 2].

Ragweed biomass is considered as a valuable energy resource. The feedstocks generated from weed biomass are sustainable sources for the production of biologically active substances (such as flavonoids, phenolic compounds, vitamins, etc.) or biogas production (methane or hydrogen). This weed can be used as a source of organic substances, in particular carbohydrates, which will ensure high efficiency in biogas production. The concentration of cellulose, lignin, and hemicellulose in the ragweed biomass ranges from 25.3% to 30.9%, 24.2% to 28.3%, and 13.1% to 17.7%, respectively. Due to the high content of these compounds, ragweed is extremely valuable for the microbial synthesis of biofuels (H_2 and CH_4 gases) [3, 4].

This work aimed to confirm experimentally the capability of ragweed degradation, methane production, and simultaneous detoxification of sewage from toxic compounds of heavy metals (copper, chromium, and iron) by a diversified microbial community. The objective was to evaluate the biotechnological potential of ragweed as a new renewable energy source.

Ragweed (*Ambrosia artemisiifolia* L.) dried biomass was used as a feedstock for CH_4 production and heavy metal immobilization. Ragweed biomass was collected in September 2022 at Sofiivska Borshchahivka village (Kyiv region, Ukraine) during active flowering.

Sludge from the sewage treatment plant in Kyiv was used as an inoculum and source of a diversified microbial community (DMC). It contained a large number of different physiological groups of microorganisms with cellulolytic activity as well as methane-producing bacteria.

To determine the patterns of *Ambrosia* degradation by a diversified microbial community (in syntrophic association), we performed five variants of the experiment: The first was the control variant (degradation of the ragweed by the native microbiome of *Ambrosia artemisiifolia* L. without using additional inoculum). The second variant was experimental (degradation of *Ambrosia artemisiifolia* L. by a diversified microbial community). The diversified microbial community was also used as an inoculum in the third, fourth, and fifth variants. Copper (II), chromium (VI), and iron (III) were inserted into different anaerobic jars in the active phase of degradation (42 days) at a concentration of 500 mg L^{-1}. The active phase of fermentation was observed on the 42nd day of degradation, during which a significant amount of anaerobic biomass was present along with a low redox potential and active methane synthesis. These conditions were favorable for the microbial immobilization of metals.

Several metabolic parameters were measured, including cumulative gas production (mL), gas phase concentration (% of CH_4 and CO_2), dissolved organic carbon concentration (DOC, mg L^{-1}), pH, and redox potential (Eh, mV).

Solutions of copper, chromium, and iron were added into the anaerobic jars (bioreactor) with tap water and *Ambrosia artemisiifolia* L. dried biomass at the 42nd hour of cultivation to the final concentrations of 100, 200, 500, and 1000 mg L^{-1} in the active phase of microbial growth. It was necessary to determine the level of resistance of the microbial community and the concentration at which maximum heavy metal immobilization would occur. Immobilization occurred in the anaerobic jars due to the active growth of a diversified microbial community (inoculum). The active phase of degradation was dominated by methanogenic microorganisms that consumed the hydrolysis products of ragweed (acetate, glucose, and hydrogen) and synthesized methane (CH_4) and carbon dioxide (CO_2). Methanogenic microorganisms actively immobilized copper compounds in the active phase of growth at concentrations of 100, 200, and 500 mg L^{-1} (Fig 1)

The efficiency of immobilization in the variants with 100, 200, and 500 mg L^{-1} Cu(II) was 100%. As expected, the fastest immobilization occurred in the presence of 100 mg L^{-1} Cu(II). The duration of complete immobilization was 6 h. Cu(II) compounds also precipitated gradually at the other concentrations. However, the immobilization durations were 12 and 30 h at 200 mg L^{-1} and 500 mg L^{-1}, respectively.

Chromium turned out to be more toxic than copper. Thus, effective immobilization occurred only in the presence of 100 and 200 mg L^{-1} Cr(VI)

The methanogenic microbial community immobilized iron with the lowest efficiency compared to copper and chromium immobilization. Methanogens reduced Fe(III) to Fe(II), which then precipitated as insoluble compounds of divalent and trivalent iron (Fig 2).

Intensive degradation of ragweed biomass was confirmed by significant methane synthesis in experimental variants where the inoculum was DMC from a sewage treatment plant. Cumulative methane production in the control was only 130 mL in the variant with DMC inoculum and under the influence of copper, it was 1120 mL and 735 mL. Thus, copper inhibited methane production by 1.5 times. Similar patterns of changes in the concentration and carbon dioxide synthesis were also observed in both the control and the experimental variants. The cumulative production of CO_2 in the control was 170 mL, in the variant with DMC inoculum, 1350 mL, and in the variant with copper, 1134 mL (Fig 3).

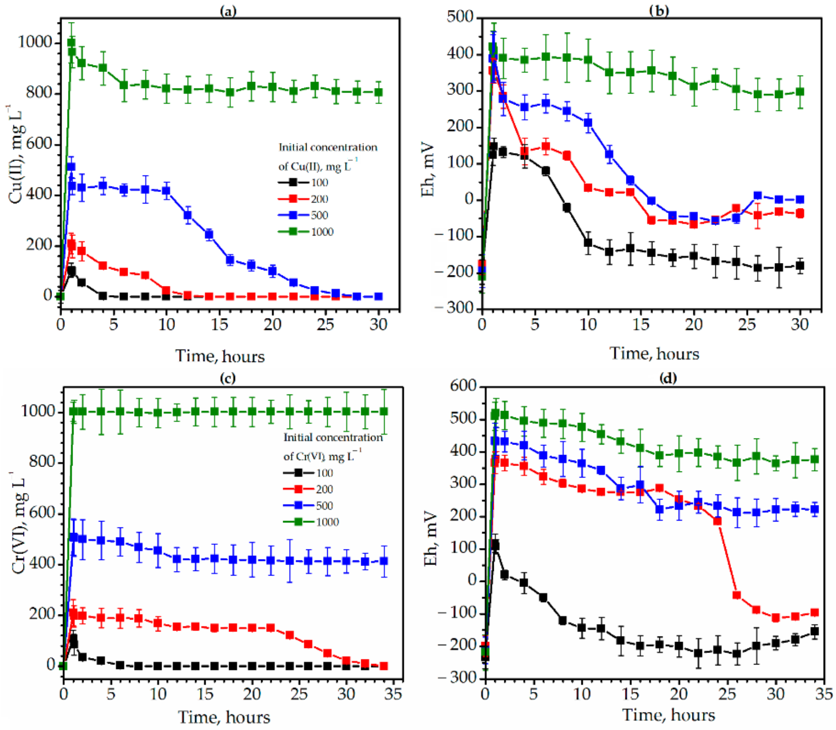

Fig 1. The dynamics of Cu(II) (**a**) and Cr(VI) (**c**) immobilization and Eh (**b**,**d**) during degradation of *Ambrosia artemisiifolia* L. weed in the presence of 100 (black lines), 200 (red lines), 500 (blue lines), and 1000 (green lines) mg L^{-1} Cu(II) (**a**,**b**) and Cr(VI) (**c**,**d**).

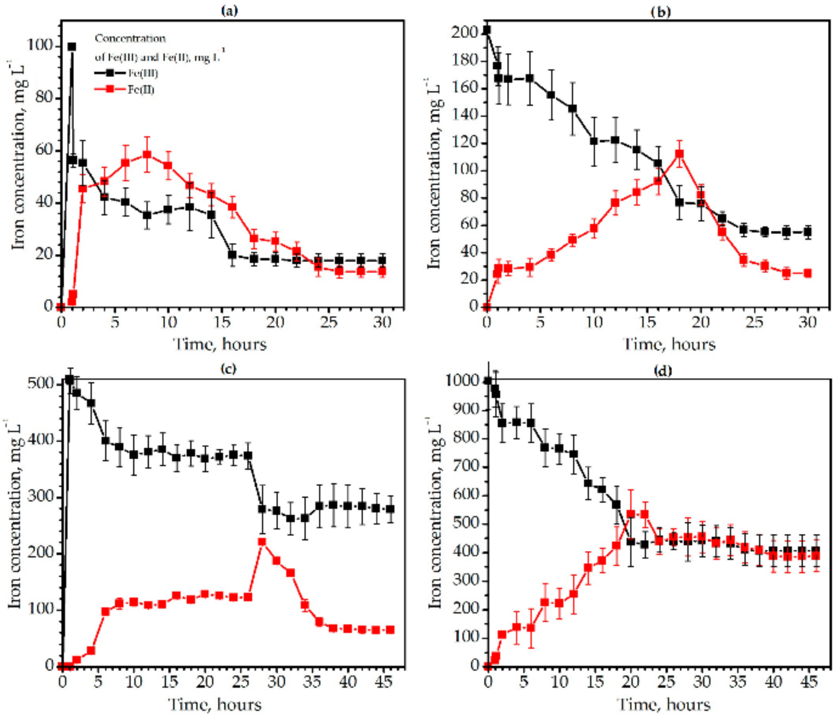

Fig 2. The dynamics of the iron (Fe (II) and Fe (III)) immobilization by the methanogenic microbial community during degradation of *Ambrosia artemisiifolia* L. weed at the initial concentrations of 100 (a), 200 (b), 500 (c), and 1000 (d) mg L^{-1} Fe (III).

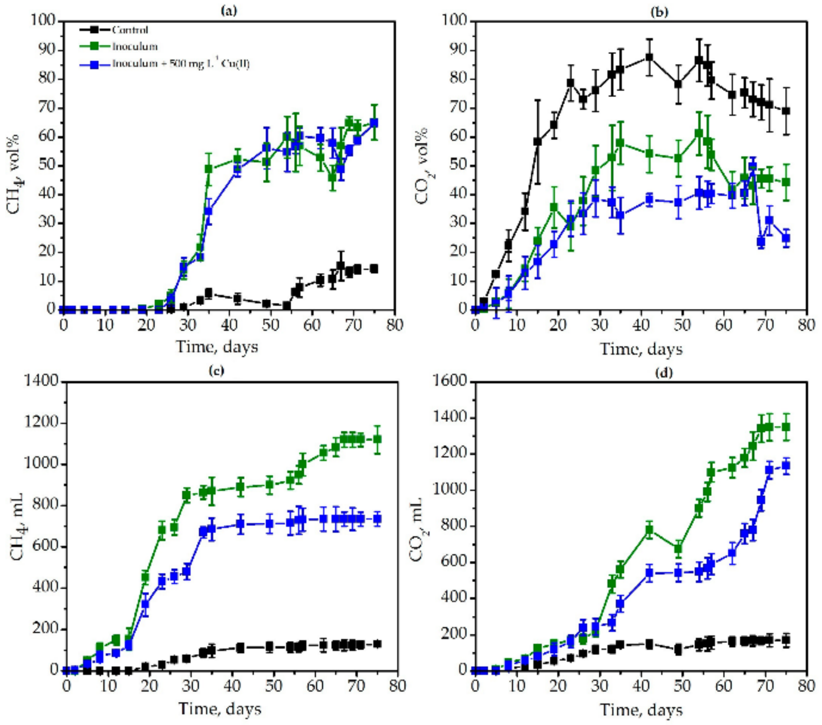

Fig 3. The dynamics of the CH_4 (a) and CO_2 (b) concentration as well as methane (c) and carbon dioxide (d) production during the degradation of *Ambrosia artemisiifolia* L. weed: the degradation in control conditions with the native microbiome (black lines), the degradation by inoculum (DMC, green lines), as well as under the influence of 500 mg L^{-1} Cu(II) (blue lines).

The methane and carbon dioxide yields during the degradation of ragweed biomass were calculated. This also confirmed the high efficiency of ragweed degradation by a diversified microbial community (Table 1).

Table 1. The effectiveness of ragweed degradation in the presence of heavy metals

Treatments	CH_4 Max, vol.%	CH_4 Yield, L kg^{-1} TS_{plant}	CO_2 Yield, L kg^{-1} TS_{plant}	K_d, Times
Control with native bacteria	14.3 ± 1.3	6.5 ± 2.2	8.5 ± 2.1	2.3 ± 0.4
Inoculum of DMC	65.1 ± 6.1	56.0 ± 7.3	67.5 ± 8.0	19.2 ± 5.4
500 mg L^{-1} Cu(II)	64.9 ± 11.3	38.4 ± 4.6	56.7 ± 9.1	11.3 ± 3.7
500 mg L^{-1} Cr(VI)	53.1 ± 11.3	22.4 ± 4.2	34.1 ± 5.4	5.3 ± 1.5
500 mg L^{-1} Fe(III)	64.3 ± 8.1	49.7 ± 5.3	68.9 ± 7.2	18.4 ± 5.2

The obtained results demonstrate the high effectiveness of using a diversified microbial community in sewage treatment plants for the degradation of *Ambrosia artemisiifolia* L., a noxious plant. Microorganisms in the syntrophic association have shown promise for the utilization of ragweed and the synthesis of biogas, as well as the complete immobilization of toxic copper compounds. Ragweed biomass was effectively degraded with methane production, indicating its capability as a sustainable feedstock for renewable energy carriers.

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