World Journal of Microbiology and Biotechnology (2024) 40:404
https://doi.org/10.1007/s11274-024-04207-4

RESEARCH

Iminoglutamic succinic acid: assessment of the degree of biodegradation and toxicity of half-decomposition products

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Received: 24 July 2024 / Accepted: 18 November 2024 / Published online: 4 December 2024

Abstract

Chelating agents used in agriculture and land reclamation practices must exhibit the capacity to firmly bind metal ions while also rapidly decomposing into non-toxic compounds due to their introduction into the environment in substantial quantities. It is therefore crucial to identify an alternative to EDTA, a prevalent chelating agent known for its low biodegradability and relatively high toxicity. This study focuses on assessing the degree of biodegradation of iminoglutamic succinic acid (IGSA) and the toxicity of its half-decomposition products. For this purpose, two bacterial and fungal isolates capable of decomposing IGSA were isolated and characterized, identified as *Ralstonia pickettii* and *Fusarium foetens*, respectively. The results of a 28-day experiment demonstrated that *Ralstonia pickettii* was capable of decomposing IGSA by 18.0% according to the manometric test and by 24.5% based on the analysis of decomposition products via HPLC. In comparison, *Fusarium foetens* exhibited a more effective biodegradation capacity, with rates of 20.3% and 32.5%, respectively. The half-decomposition products of IGSA were characterized by low ecotoxicity levels (LID10) concerning *Paramecium caudatum* (11.2–13.2 g/l) and *Ceriodaphnia affinis* (3.6–8.9 g/l). However, a stimulating effect was observed on microalgae, with growth stimulation ranging from 63.65–96.60%.

Keywords Chelates · Iminoglutamic succinic acid · Micronutrients · Ecotoxicity · Biodegradation

Introduction

The increasing population has resulted in an elevated demand for high-quality agricultural products. The primary factor affecting the yield of agricultural crops and the quality of products is soil condition, particularly the level of its fertility (Kaftan and Zenkova 2019). Soil fertility depends on several factors, including soil type, biological activity, structure, tillage methods, and the improvement of agrochemical and physical properties (Vejan et al. 2016; Dridiger et al. 2017; Steponavičienė et al. 2023). Currently, soil productivity and the quality of agricultural products are declining due to overexploitation of soil resources and various additional factors (Ye et al. 2020; Mishina and Surova 2020). Consequently, experts are investigating methods to preserve soil health and enhance yield.

The application of fertilizers, including nitrogen, phosphorus, and potassium, represents a highly effective method for achieving optimal fertility and improving crop quality. Nevertheless, the long-term maintenance and enhancement of this outcome are feasible through the incorporation of micronutrients based on chelated compounds. Trace elements are essential for plants as they participate in various physiological processes, including enzyme activity, development and growth, and the maintenance of normal reproductive functions (Kheir et al. 2021; Brusko et al. 2023). Chelated compounds are designed to enhance the bioavailability of trace elements, thereby increasing their assimilation efficiency by plants, which in turn leads to enhanced productivity and biomass (Murali et al. 2018; Brusko et al. 2023). Chelating agents also play a significant role in both the medical and environmental

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protection fields, with a high level of production and adoption across various applications. This, in turn, results in increased ingress and accumulation in different components of the environment. However, the accumulation of chelating agents in the soil increases the mobility of heavy metals, which consequently enhances their toxic effects (Huang et al. 2019). Furthermore, plants can accumulate a range of heavy metals It is therefore essential that chelating agents entering the environment possess the capacity for rapid degradation while ensuring that their decomposition products are non-toxic.

Currently, many widely used chelated compounds are available—salts of DTPA (diethylenetriaminepentacetic acid), EDTA (ethylenediaminetetraacetate acid,), OEDP (oxyethylidenediphosphonic acid), IDS (iminodisuccinic acid) (Nörtemann 1999; Geiger et al. 2017; Brusko et al. 2023). A review of the literature reveals that the use of micronutrients based on these compounds to increase yields is associated with certain concerns. Several chelating agents possess characteristics that have a detrimental impact on the environment when employed. For instance, EDTA and its associated compounds are not amenable to biological degradation, and photochemical decomposition is a relatively slow process (Nörtemann 1999). High concentrations of EDTA have been shown to possess toxic properties for both plants and microbiota (Canuel et al. 2021). The chelating properties of the compound result in the remobilisation of metal ions (including heavy metals), active assimilation by organisms and an increase in vertical migration of elements and pollution of ecosystem components (Farberova et al. 2012; Wu et al. 2021). The high binding capacity of EDTA concerning metals results in a reduction in the extent of biodegradation of these complexes (Bloem et al. 2017). Additionally, numerous chelating agents used in agricultural applications have the potential to contribute to the leaching of metals from soils, which may ultimately lead to the depletion of these elements.

In light of the aforementioned characteristics of currently used chelating compounds, there is a need to use more environmentally friendly materials for the production of micronutrients, given that this type of fertilizer can provide crops with the requisite mineral nutrition. It is crucial to consider the characteristics and evolving behavior of these compounds when they are introduced into the soil.

The staff of the "Materials for Green Energy and Sustainability" laboratory at the Chemical Institute of Kazan Federal University have synthesized a substance with chelating properties—iminoglutamic succinic acid (IGSA). Additionally, in collaboration with the staff of the Institute of Ecology and Environmental Management at Kazan Federal University (Beltyukova et al. 2023; Brusko et al. 2023), the effectiveness of this substance as a chelating agent in vegetation experiments was demonstrated, along with its low toxicity and high degree of biodegradation. The substance is believed to offer a more environmentally friendly alternative to EDTA.

Nevertheless, prior to the commercialisation of a compound, it is essential to ascertain the mechanisms of its degradation and the nature of the biodegradation products, in addition to evaluating its toxicity. The objective of this study was to identify the microorganisms that cause the degradation of IGSA and to evaluate the toxicity of the resulting biodegradation products.

Materials and methods

The studied compound

The work used a compound – iminoglutamic succinic acid, which was obtained at the Butlerov Chemical Institute of KFU in accordance with the methodology previously outlined (Brusko et al. 2023).

Isolation of microorganisms that degrade IGSA

This study examined microorganisms that utilize IGSA as a carbon source. The microorganisms were isolated from a hydroponic solution that had been used in an experiment to evaluate the fertilizing properties of IGSA containing manganese and zinc salts. To isolate the microorganisms, 100 µL of solution was placed into Petri dishes containing Bushnell-Haas agarized medium (KH_2PO_4 – 1 g/L; K_2HPO_4 – 1 g/L; NH_4NO_3 – 1 g/L; $MgSO_4 \cdot 7H_2O$ – 0.2 g/L; $FeCl_3$ – 0.05 g/L; $CaCl_2$ – 0.02 g/L) with the addition of the sodium salt of IGSA ($Na_{3,5}$IGSA) as the sole carbon source. The dishes were incubated at 28 °C for a period of 48 h. The sodium salt of IGSA was selected for use in this experiment due to the widespread occurrence of this metal in nature and its relatively low toxicity compared to other micronutrients.

In order to identify the microorganisms that most actively consume $Na_{3.5}$IGSA as a carbon source, two selection techniques were employed: the weight method for micromycetes (Farberova et al. 2020) and the growth curve method for bacterial cultures (Rogers et al. 2022). The mycelium of the micromycete was inoculated with an inoculation loop into a liquid BH medium containing $Na_{3,5}$IGSA. The growth of micromycetes was conducted in glass conical flasks with a volume of 250 mL, with the growth medium volume set at 100 mL. The flasks were incubated in a shaker incubator set at 28 °C with a shaking speed of 114 rpm for a period of 72 h. The mycelial biomass was separated from the liquid by centrifugation at room temperature (3500 rpm for 10 min), after which it was dried at 40 °C to a constant weight.

In order to obtain bacterial growth curves, precultures were grown in BH medium with $Na_{3.5}$IGSA. The overnight

culture was prepared in 250 mL glass conical flasks, with a growth medium volume of 100 mL. A total of 180 µL of pure BH medium and 20 µL of the overnight culture were introduced into the wells of a 96-well microplate. The optical density was measured for a period of 72 h using a Multiskan FC microplate photometer (Thermo Fisher Scientific Inc., USA) with a wavelength of 595 nm (measurement mode: t = 28 °C, with a 5-s shaking cycle every 30 min prior to measuring the optical density).

Characteristics of $Na_{3,5}$IGSA destructors

To evaluate the expansion of the colony, micromycetes were cultivated on Czapek's agar (Biocompas-S, Russia). The colony diameter was measured at 24-h intervals over a seven-day period. To characterise the growth of the bacterial colony, growth curves were constructed on LB medium (g/L: peptone–10; yeast extract–5; sodium chloride–10). The overnight culture was prepared in 250 mL glass conical flasks, with a growth medium volume of 100 mL. A total of 180 µL of pure LB medium and 20 µL of the night bacterial culture were introduced into the wells of a 96-well microplate. The optical density was determined over a 24-h period using a Multiskan FC microplate photometer with a wavelength of 595 nm (measurement mode: t = 28 °C, with a 5-s shaking cycle every 30 min prior to measuring the optical density).

Microscopic examination of the isolates was conducted using a ZEISS Axio Scope.A1 light binocular microscope (ZEISS, Germany) under transmitted light. To ascertain the Gram group of the bacterial isolate, a Gram staining reaction was peformed. A small piece of the bacterial culture was placed onto the center of a clean microscope slide, spread across the slide, and dried over an alcohol burner flame for staining and microscopic examination. A small piece of the fungal culture was placed onto the center of a clean microscope slide with a drop of distilled water, spread across the slide, and covered with a coverslip for microscopic examination. The genus of the micromycete was determined through morphological analysis, referencing a guidebook for the identification of fungal species (Bilai and Kurbatckaya 1990). The description of bacterial and fungal colonies was carried out using morphological criteria (Sousa et al. 2013; Yakuba et al. 2019).

The Biolog Ecoplate™ was used to analyze the physiological profile of isolates (Németh et al. 2021). The preculture was cultivated for 72 h in glass conical flasks with a volume of 100 mL in BH medium containing $Na_{3,5}$IGSA (the medium volume was 30 mL). The culture medium was diluted with a sterile BH medium to achieve a cell concentration of 3.2×10^3 CFU/mL. A volume of 150 µL per well of this medium was introduced to the Biolog Ecoplate using a multi-channel pipette. The microplate was then incubated in the dark for 24 h at a temperature of 28 ℃. The optical density was determined at 24-h intervals over a five-day period.

The identification of the isolates was conducted through Sanger sequencing, a process carried out by specialists at SPC "Syntol" in Moscow, Russia. Bacterial and fungal DNA were extracted using the FastDNA® SPIN Kit for soil (MP Biomedicals, 29,525 Fountain Parkway, Solon, OH 44139, California, US) following the manufacturer's instructions.

Measurement of substrate consumption rate

To evaluate the intensity of biodegradation and analyse the composition of the resulting products, an experiment was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals (Method 301F) (OECD 1992) using the WTW™ OxiTop™ Measuring Vessel. The preculture was cultivated in the BH medium with $Na_{3,5}$IGSA for 48 h at a temperature of 28 °C and a shaking speed of 114 rpm. A 2 mL sample of the preculture was introduced into 150 mL of growth medium (BH medium with $Na_{3,5}$IGSA) within the OxiTop vessels. Aniline was used as a control substance. To eliminate the possibility of self-decomposition of $Na_{3,5}$IGSA, the BH medium with $Na_{3,5}$IGSA was used without inoculum. The concentration of $Na_{3,5}$IGSA was 20 g/L, while that of aniline was 8.5 g/L, resulting in an identical final carbon concentration. The vessels were incubated in the dark for 28 days with shaking at 26 rpm and a temperature of 28 °C.

The intensity of the biodegradation process was determined by calculating the ratio of biochemical oxygen demand (BOD) to theoretical oxygen demand (ThOD) (Brown et al. 2018).

BOD was calculated by using this equation:

$$\mathrm{BOD}\left(\mathrm{mg\ L^{-1}}\right) = \frac{MO_2}{RT_{28}}\left(\frac{\mathrm{V_{TOTAL}} - \mathrm{V_{LIQUID}}}{\mathrm{V_{TOTAL}}} + \alpha\frac{T_{28}}{T_0}\right)\Delta p(O_2)$$

where:

MO_2 is the molecular weight of oxygen (32,000 mg mol^{-1}).

R is the gas constant (83.144 mbar mol^{-1} K^{-1}).

T_0 is the temperature at 0 ℃ (273.15 K).

T_{21} is the measuring temperature (301.15 K).

V_{TOTAL} is the total volume of the test vessel (500 mL).

V_{LIQUID} is the liquid volume in the test vessel (150 mL for the control samples, 152 mL for the tested samples).

α is the Bunsen Absorption coefficient (0.03103).

Δ $p(O_2)$ is the difference of the partial pressure of the oxygen, measured as a drop in pressure in the test vessel (173.5 hPa, 193.5 hPa, 19 hPa for bacteria, micromycetes and control, respectively).

The ThOD was calculated using the following equation:

$$ThOD = \frac{16[2C + 0.5H - O]}{MW}$$

where:

C, H and O are the number of carbons, hydrogens and oxygens in the elemental formula of the test substrate (C – 9, H – 13, O – 8);

MW is the molecular weight of the test substrate (329 g mol^{-1}).

Toxicity assessment of biodegradation products

The toxicity of the biodegradation products was determined using test organisms: *Paramecium caudatum*, *Ceriodaphnia affinis* (Lavorgna et al. 2016; Kuryntseva et al. 2016) and *Chlorella vulgaris* (Selivanovskaya et al. 2023). The toxicity of the samples was evaluated immediately after sampling, following the conclusion of the biodegradation experiment. A series of dilutions of the samples was prepared, comprising two test dilutions for *P. caudatum* (1, 1:1) and four test dilutions for *C. affinis* (1, 1:1, 1:2, 1:3), as well as a single dilution for tests with *C. vulgaris* (1:10). All variants of the experiment were conducted in two replicates or more.

The *P. caudatum* tests were conducted in a microplate containing 0.3 mL/well of the test solution at a temperature of 20 ± 2 °C. Ten organisms were placed in each well, and the plates were observed with a stereoscopic digital microscope, the Micromed MS-2 ZOOM (NEW TECHNOLOGIES GROUP, Moscow, Russia). Mortality was determined by counting the number of surviving organisms after one hour of the experiment.

In order to ascertain the toxicity of each sample, a series of tests were conducted using *C. affinis*. Each sample was

Table 1 Cultural features of the fungi isolate (genus *Fusarium*)

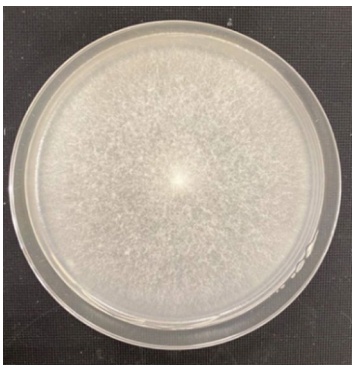
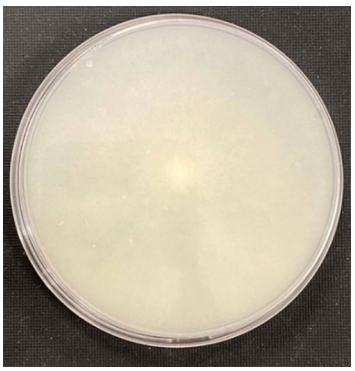
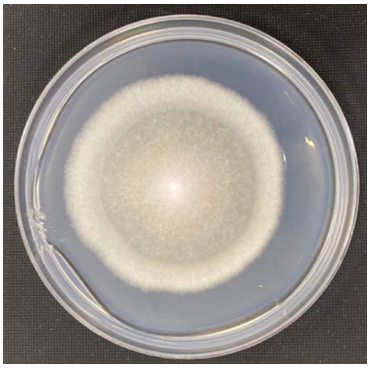
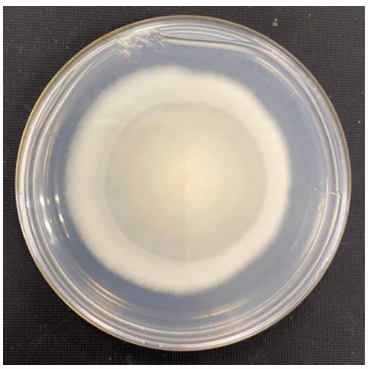
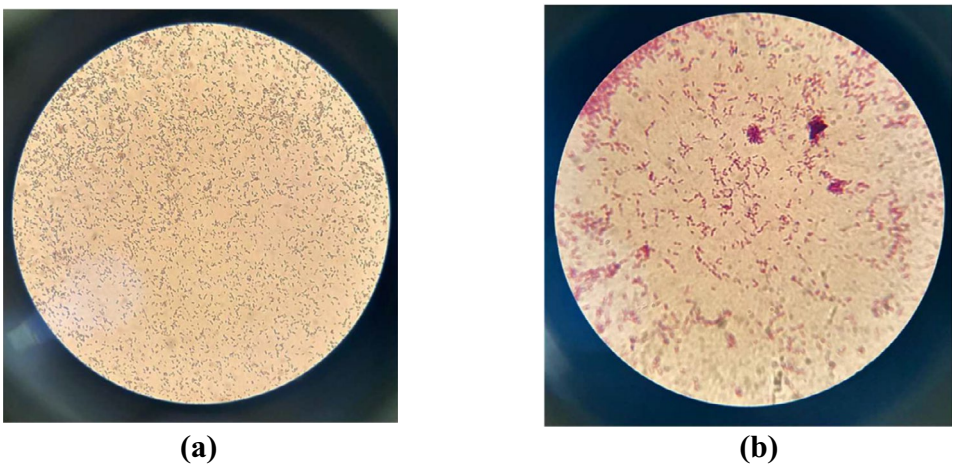
Isolate	Morphological and cultural features of the colony	Variations of features
Micromycete cultivated on Chapek medium	Colony texture	Fluffy
	Colony surface	Smooth
	Colony colour	White
	Colony underside	White
	Colony form	Circular
	Colony margin	Villous
	The central part of the colony	Flat
	Mycelium	Aerial
	Mycelium colour	White

Table 1 (continued)

Micromycete cultivated on Chapek medium with 40% lactic acid  	Colony texture	Fluffy
	Colony surface	Smooth
	Colony colour	Concentric, gray in the center, white towards the periphery
	Colony underside	Concentric circles, gray in the center, white towards the periphery
	Colony form	Curcular
	Colony margin	Villous
	The central part of the colony	Convex
	Mycelium	Aerial
	Mycelium colour	White



(a) **(b)**

Fig. 1 Photo of the bacterial isolate: **a** 640 × zoom; **b** 1600 × zoom

diluted in triplicate, with control samples also included. A volume of 50 mL of the tested samples was poured into the vessels, and five organisms were placed therein. The vessels were incubated for 48 h in a climatostat at a temperature of 20 ± 2 °C, with a 12:12 light cycle. Following this period,

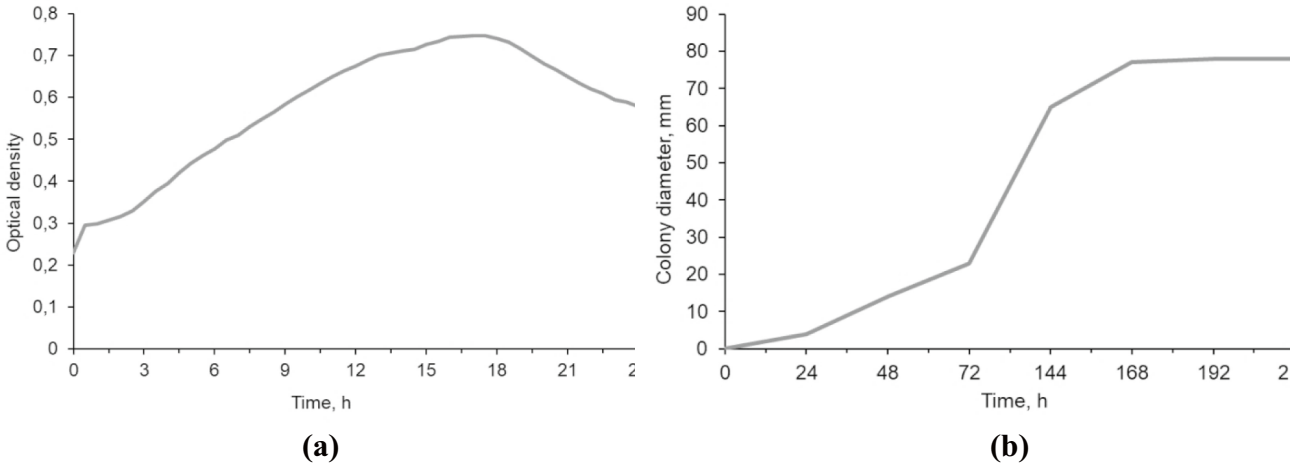


Fig. 2 Microorganism growth curves: **a** *R. pickettii*; **b** *F. foetens*

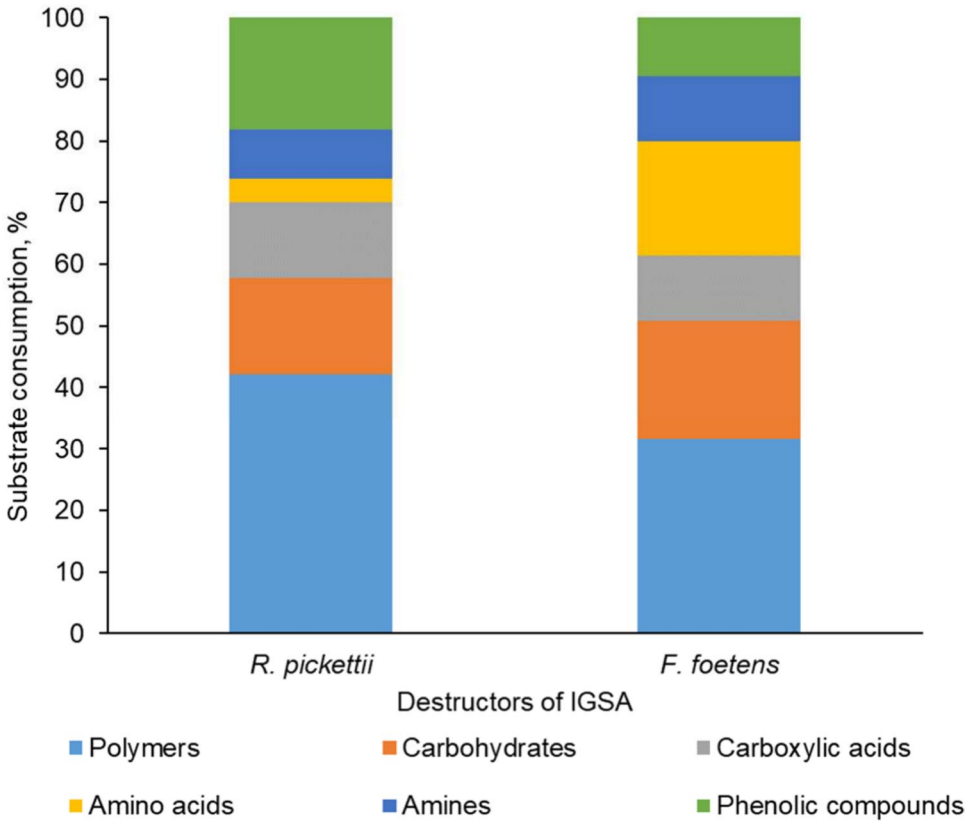


Fig. 3 Substrates consumption by destructors of $Na_{3,5}$IGSA

the number of surviving organisms was estimated (Terehova et al. 2017).

For tests with *Chlorella vulgaris*, an algae culture was prepared on Tamiya medium (per 1 L of H_2O: 2.5 g KNO_3, 1.25 g $MgSO_4*7H_2O$, 0.625 g $KH_2PO_4*3H_2O$, 0.0015 g iron citric acid, 0.5 ml of trace element solutions), and its optical density was measured and adjusted to a value of 0.125 by diluting with Tamiya medium. A negative control was conducted using distilled water. The prepared test culture of algae was introduced (in a volume of 2 mL) into a glass vessel containing 48 mL of the control and test samples. The samples were then transferred into 6 mL tubes and covered with clean polyethylene lids with 6 mm holes. Subsequently, the sealed tubes were positioned within a multicuvette algae cultivator (KVM-05, TU 26.51.70–001-63790925–2021) for a period of 22 h at a temperature of 36 ± 0.5 °C and an average light intensity

Table 2 The rate of biodegradation of IGSA determined by the results of manometric analysis and HPLC

Organism	*R. pickettii*	*F. foetens*
BOD, mg/L	161.91	180.58
ThOD, mg/mol	802.43	
BOD (reg. ThOD), %	17.96*	20.28*
Content of IGSA in samples after the experiment, mg/mL	7.55	6.75
Degradation of IGSA, %	24.50*	32.50*

*p-value < 0.05

Table 3 LID10 values for *P. caudatum*, *C. affinis* and *C. vulgaris* growth stimulation indices during Na3.5IGSA biodegradation for each of the repeats ($Na_{3.5}$IGSA is the sodium salt of IGSA, B–$Na_{3.5}$IGSA is the sodium salt of IGSA with a bacterium, F-$Na_{3.5}$IGSA is the sodium salt of IGSA with a micromycete)

Sample	LID10 (*Paramecia caudatum*), g/L	LID10 (*Ceriodaphnia affinis*), g/L	Growth stimulation (*Chlorella vulgaris*), %
Na3,5IGSA	11,00	3,30	53,80
B-Na3,5IGSA	11,20	3,60	63,65
F-Na3,5IGSA	13,20	8,90	96,60

of 60 W/m^2. Following a 22-h incubation period, the optical density was determined using a spectrophotometer (PE5300vi) in a 10 mm thick cuvette at λ = 670 nm (Selivanovskaya et al. 2023).

In order to ascertain the toxicity of the test samples, the LID10 value was calculated for each sample for *P. caudatum* and *C. affinis*. In the case of tests with *C. vulgaris*, the toxicity was calculated based on the relative difference in the values of the indicators in the experimental variants compared with the control. The criterion for toxicity was the suppression of algae culture growth by 20% or more, or the stimulation of growth by 30% or more.

Analysis of products composition before and after biodegradation of IGSA

The composition of the products was analysed before and after biodegradation of IGSA in the presence of bacteria and fungi using an ACQUITY UPLC I-Class Core Sys w/SM-FTN high-pressure liquid chromatograph combined with a Xevo TQD Triple Quadrupole Mass Spectrometry detector, which was equipped with an ACQUITY UPLC BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm × 50 mm. During the analysis, a solution of water and acetonitrile (in a ratio of 95:5) was used as the eluent, with the addition of ammonia as a buffer (0.06%). The analysis settings were isothermal, with a temperature of 30 °C, an eluent flow rate of 0.05 ml/min, nitrogen used as a desolvation gas, electrospray ionisation in the positive mode, and full ion scanning. The analysis was conducted in triplicate for each sample.

Statistics

All of the experiments were performed in triplicate. Statistical analysis was conducted using the R free software environment (version 4.4.1). The datasets were evaluated for normal distribution using the Shapiro–Wilk test at the 0.05 significance level. In instances where the null hypothesis of normal distribution was not rejected, the Fisher test at the 0.05 significance level (var.test) was used to assess the homogeneity of variances. In cases where the variances were homogeneous, the Student's t-test was used to determine the significance of differences between the means of the datasets. In the event that at least one of the datasets under comparison exhibited a non-normal distribution, the non-parametric Mann–Whitney test (wilcox.test) was employed to ascertain the significance of the observed differences between the sample means.

Results

Description of microorganisms

In the initial stage of the study, a description of the bacterial and fungal isolates selected for research based on substrate consumption intensity data was conducted. According to microscopy data, the fungal isolate was identified as belonging to the genus *Fusarium* (Bilai and Kurbatckaya 1990). The description of the micromycete, based on its morphological and cultural characteristics (Yakuba et al. 2019) is presented in Table 1.

Microscopic examination of the bacterial isolate and Gram staining revealed that the isolate belonged to the group of Gram-negative bacilli (Fig. 1).

A description of the bacterial colony was provided (Sousa et al. 2013). The bacterial colony is white, semi-transparent, and has a mucilaginous consistency; it is circular in shape, with a smooth margin and a convex profile, and its structure is homogeneous.

The results of the identification of isolates indicated that the bacteria belonged to the genus *Ralstonia* with a probability of 95.20% that the isolate belonged to the species *Ralstonia picketii* (Appendix 1). The determination of the micromycete belonging to the genus *Fusarium* was confirmed with a probability of 96.09% that the isolate belonged to the species *Fusarium foetens* (Appendix 1).

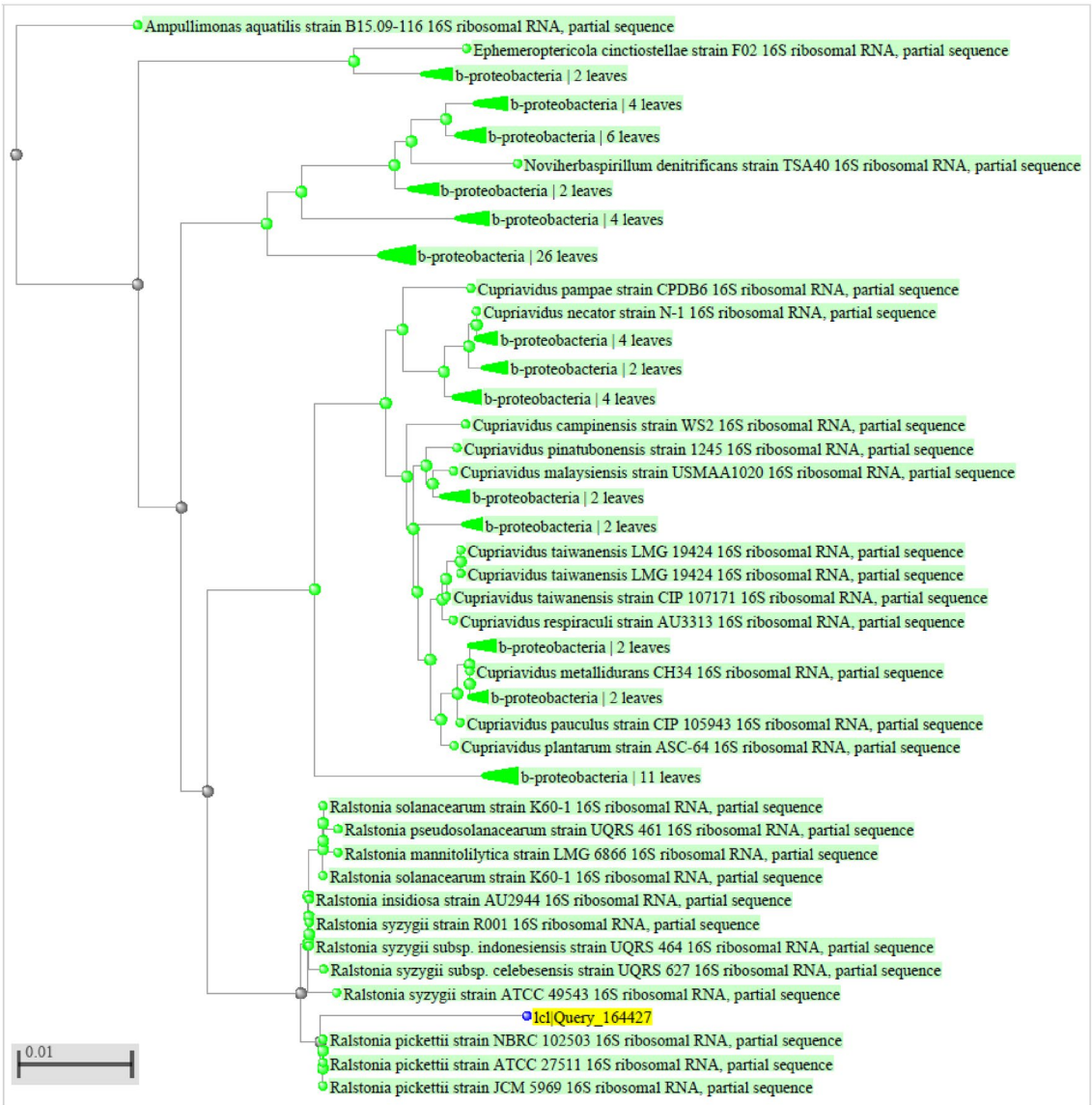


Fig. 4 Phylogenetic tree of bacterial isolate (**A**) and micromycete (**B**)

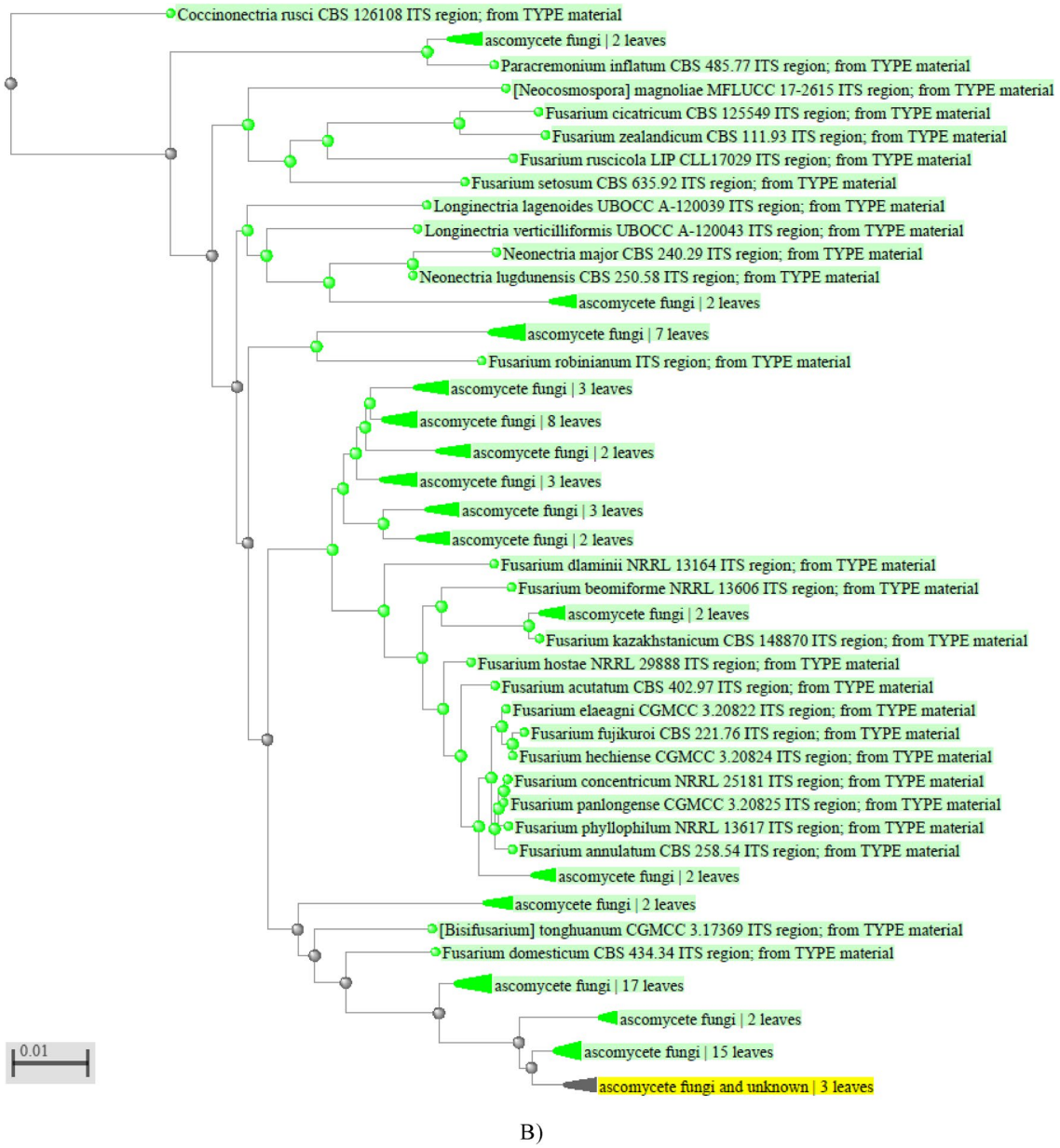
Microorganism growth curves

In order to assess microbial growth, the growth curves for *R. pickettii* and *F. foetens* were plotted (Fig. 2). The growth rate of the bacterial isolate can be characterised as low, as evidenced by the prolonged exponential phase. The peak growth activity of *R. pickettii* is observed at the 17th hour of incubation, after which the optical density reaches a plateau. *F. foetens* exhibited a standard growth rate, with the mycelium occupying the entire surface of the Petri dish after six days of cultivation.

Specificity of carbon substrates consumption

The results of the physiological profile analysis of the isolates enabled the calculation of the average well color development index (AWCD) based on data obtained from the consumption of carbon substrates. The AWCD index for *F. foetens* was found to be 0.32, indicating a higher level of metabolic activity. In contrast, *R. pickettii* exhibited a lower AWCD index of 0.26, suggesting reduced metabolic activity relative to the studied substrates.

Figure 3 illustrates the extent of substrate consumption by *F. foetens* and *R. pickettii*. This enabled us to ascertain that both organisms possess the capacity to utilise the substrates



B)

Fig. 4 (continued)

of all the aforementioned groups to varying degrees. However, significant differences (p-value < 0.05) in the degree of substrate consumption, based on statistical analysis of the data, were observed only for amino acids. *F. foetens* demonstrated a 14.7% higher intensity of amino acid consumption compared to *R. pickettii*. Both microorganisms exhibited a distinct preference for polymers among the substrates – 42% for *R. pickettii* and 32% for *F. foetens* (Fig. 3).

Biodegradation of $Na_{3.5}$IGSA and Its product's toxicity

Table 2 was constructed based on the results of the biodegradation calculations for the studied substance, as reflected in the BOD and ThOD data (Table 2):

The biodegradation process carried out by *F. foetens* was observed to reach an intensity of 20%. *R. pickettii* demonstrated an efficiency of approximately 18% in decomposing $Na_{3.5}$IGSA. The analysis of the products' composition before and after the biodegradation of $Na_{3.5}$IGSA revealed that the bacterial isolate decomposed the compound to a lesser extent

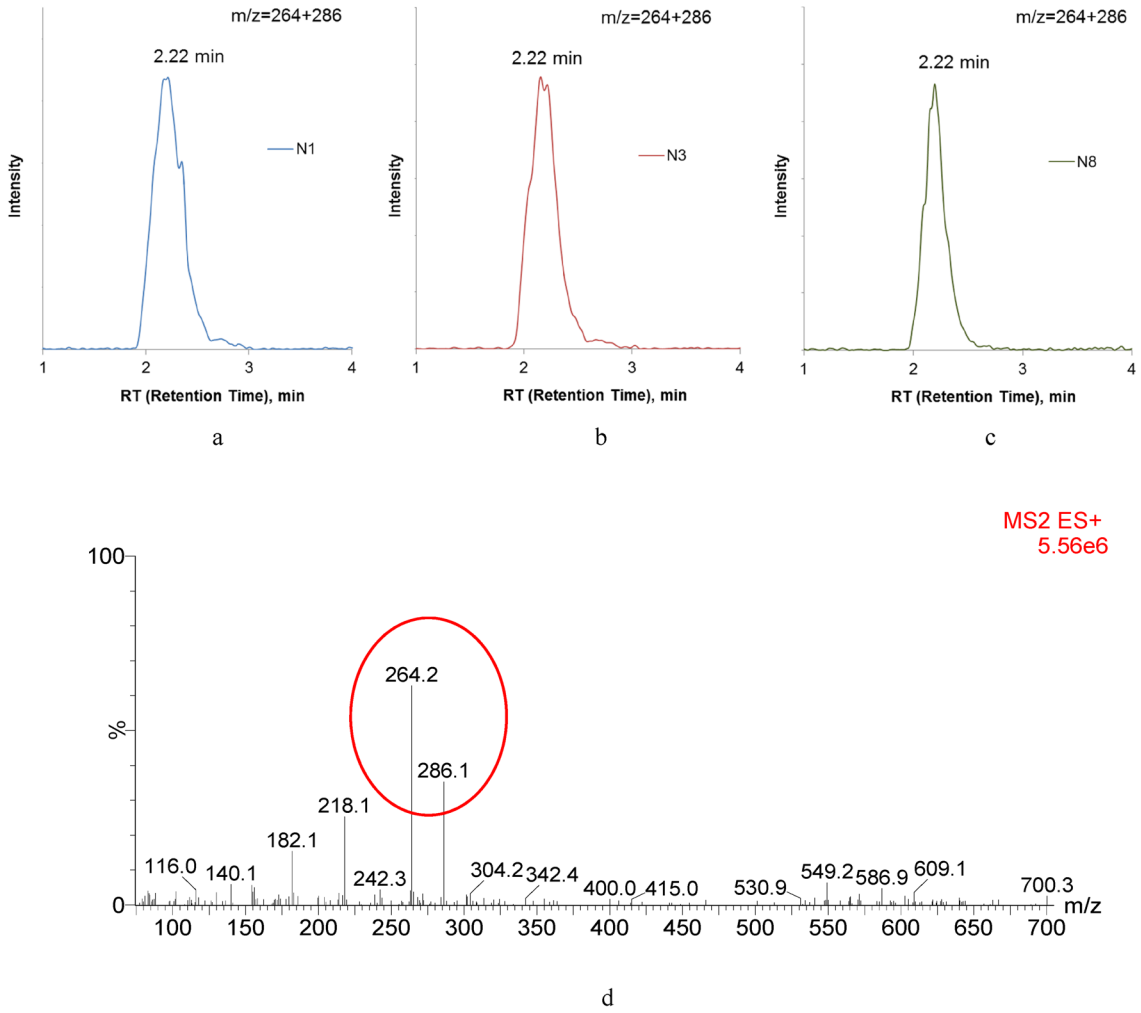


Fig. 5 Chromatograms of formations $Na_{3,5}$IGSA in the middle of BH (**a**), $Na_{3,5}$IGSA + *R. pickettii* in BH (**b**), $Na_{3,5}$IGSA + *F. foetens* in BH (**c**) and the mass spectral characteristic with RT = 2.22 min (**d**)

than the fungal one. The analysis demonstrated a reduction in the concentration of $Na_{3,5}$IGSA when decomposed by *R. pickettii* by 24.50%, whereas the activity of *F. foetens* allowed for a decrease in the concentration of this compound by 32.50% (Appendix 2). The results of the manometric analysis and high-performance liquid chromatography correlate with each other. The discrepancy in the biodegradation values obtained from different analyses can be explained by differences in the techniques used, as the methods yield different values. High-performance liquid chromatography (HPLC) is designed to determine the concentration of the test substance in the obtained mixture. The biodegradation was calculated by subtracting the remaining amount of $Na_{3,5}$IGSA, as determined by HPLC, from the initial concentration of $Na_{3,5}$IGSA added in the beginning of the experiment (Takekoshi et al. 2022). The manometric test, in turn, records the progress of the biodegradation process by monitoring changes in carbon dioxide pressure, which serves as an indicator of substance decomposition (Vähäoja et al. 2005). Based on this value, the degree of biodegradation of the substance is calculated. It should be noted that complete mineralisation of the substance to carbon dioxide may not occur, as microorganisms can consume the substance only up to a certain degree, thereby carrying out only one stage of degradation (Reineke 2001). In this context, the biodegradation values obtained by manometric analysis were found to be lower than those obtained from the HPLC method.

The chelating agent currently most widely used in many fields, EDTA, is the subject of numerous studies regarding the biodegradation of compounds and their toxicity. For instance, the extent of EDTA biodegradation was previously determined to be 6.5%, which was found to be approximately threefold less than the values obtained for IGSA (Beltyukova et al. 2023). However, the decomposition of these substances was carried out by the microbial community as a whole, rather than by individual strains. It is hypothesised that

biodegradation will occur at a lower rate at the individual strain level. However, the objective of this study was to characterise the biodegradation products, therefore individual strains were selected for analysis rather than a microbial community. In general, the data indicate that the expected average degree of biodegradation for IGSA is 802.43 and 171.24 mg/l, respectively.

Assessment of the ecotoxicity of $Na_{3.5}$IGSA

The subsequent stage of the study was to evaluate the toxicity of $Na_{3.5}$IGSA and its decomposition products in relation to hydrobionts (Table 3). The results demonstrate that the studied compound and its biodegradation products are non-toxic to *P. caudatum* and *C. affinis*. However, it has been observed that the compound can stimulate the growth of *C. vulgaris*, with the greatest intensity of stimulation occurring under the influence of *F. foetens* activity. On average, growth stimulation exceeds 90% of the control values.

Discussion

Beltyukova et al. obtained data on the toxicity of individual salts of widely used EDTA complexes (Beltyukova et al. 2023). For instance, when LID10 was found to be equal to or greater than 1 g/L, the compound was deemed non-toxic. In contrast, the toxicity of EDTA was observed to be 1.4 and 0.3 g/L in tests with *C. vulgaris* and 0.2 and 0.4 g/L in tests with *D. magna*. Similarly, comparable results were obtained when assessing the toxicity of complexones and chelates using alternative test organisms (Sillanpää 1997; Oviedo and Rodríguez 2003). Furthermore, it has been demonstrated that chelating substances, such as EDTA and their derivatives, can accelerate the eutrophication of aquatic systems and promote the proliferation of microalgae (Sillanpää 1997; Bucheli-Witschel and Egli 2001).

A synthesis of the data obtained and the existing literature leads to the conclusion that the studied compound is non-toxic to hydrobionts, but has the potential to stimulate the growth of microalgae. The results of the tests conducted on *P. caudatum* and *C. affinis* demonstrated that the compound exhibited disparate effects on the two organisms. In comparison with *P. caudatum*, the test with *C. affinis* indicated a greater degree of toxicity (as shown by a lower value of LID10). However, in general, the effect of the compound cannot be defined as toxic. It is worth noting that the mean percentage of biodegradation of the compound during the experiment was only approximately 19%, which may potentially influence the toxicity of the biodegradation products. Furthermore, the compound has been observed to stimulate the growth of *C. vulgaris*, with the greatest intensity occurring under the influence of micromycete activity. On average, growth stimulation exceeds 90% of the control values. It can be concluded that the toxicity of the compound and its degradation products depends on several factors, including the destructive organism and its substrate specification (Taştan et al. 2017), the test organism (Lavorgna et al. 2016; Gueretz et al. 2017), and the conditions under which the decomposition process occurs (Kováts et al. 2002).

Conclusion

Before a new substance can be used, it is necessary to assess its toxicity. However, it is equally important to evaluate its degradation in various components of the environment—soil and water—and to assess the products of that degradation. Iminoglutamic succinic acid is a new chelate-forming compound, and the aim of this work was to evaluate its degradation under controlled conditions. The isolated microorganisms carry out part of the biodegradation process for this substance since they were isolated using a medium containing the test substance as the sole carbon source. Our study showed that $Na_{3.5}$IGSA is less toxic compound to aquatic organisms than EDTA. The biodegradation rate of $Na_{3.5}$IGSA is higher than that of EDTA. Therefore, if $Na_{3.5}$IGSA enters environmental components, it is unlikely to accumulate in quantities that would negatively impact the biota of water, soil and other natural environments. Additionally, the possibility of $Na_{3.5}$IGSA degradation by soil and aquatic organisms has been demonstrated.

To gain a comprehensive understanding of this substance behaviour in a natural environment, it is essential to study its semi-degradation products, which may exhibit higher toxicity and persistence in environment. It is thus imperative to conduct further research on $Na_{3.5}$IGSA with the aim of characterising its semi-degradation products.

Appendix

See Appendix (Figs. 4 and 5).

Acknowledgements the work is carried out in accordance with the Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University of the Government of the Russian Federation.

Author contribution All authors contributed to the study conception and design. P.A. is the author of the research idea, she was engaged in the distribution of work tasks, supervising, editing the final manuscript. N.A. was engaged in work related to the isolation and identification of strains, analyzing the degree of biodegradation using a manometric test

and identification of strains, calculating the results obtained, made a major contribution to the writing of the article and its translation and editing the final manuscript. Y.O. performed work on isolating strains, performing a manometric test, constructing growth curves, identifying strains, performing toxicity tests, formatting the results of the manuscript. D.L. was engaged in performing a test for the analysis of the physiological profile of isolates, was engaged in work related to the construction of growth curves of isolates. A.R., A.N. and M.M. performed work related to the analysis of the composition of the products of semi-decomposition of IGSA before and after biodegradation using HPLC and calculating the results. P.Y. and S.Y. provided general guidance, searched of the funding, took part in the discussion of the results and the final version of the work.

Funding The work is carried out in accordance with the Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University of the Government of the Russian Federation.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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