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Hygienically optimised collection of biowaste with ecovio biowaste bags

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1 Introduction

The food fit for human consumption produced from agricultural products does not contain any health-relevant quantities of human-pathogenic microorganisms. Many foods are mostly stored chilled until they are prepared for consumption, in order to stop germs from multiplying. However, the waste of such food goes off very quickly at room temperature. During handling, opportunistic and/or inevitable pathogens can pass from sick persons into the biowaste (organic waste) where they multiply rapidly at typical living space temperatures. Therefore, the biowastes delivered to biowaste treatment plants are deemed to be cause for concern or rather risky in epidemic and phytohygienic terms. Compliance with minimum process requirements during the biowaste treatment (composting, fermentation) ensures that hygienically risky biowastes can be recovered as epidemiologically and phytohygienically safe products[1].

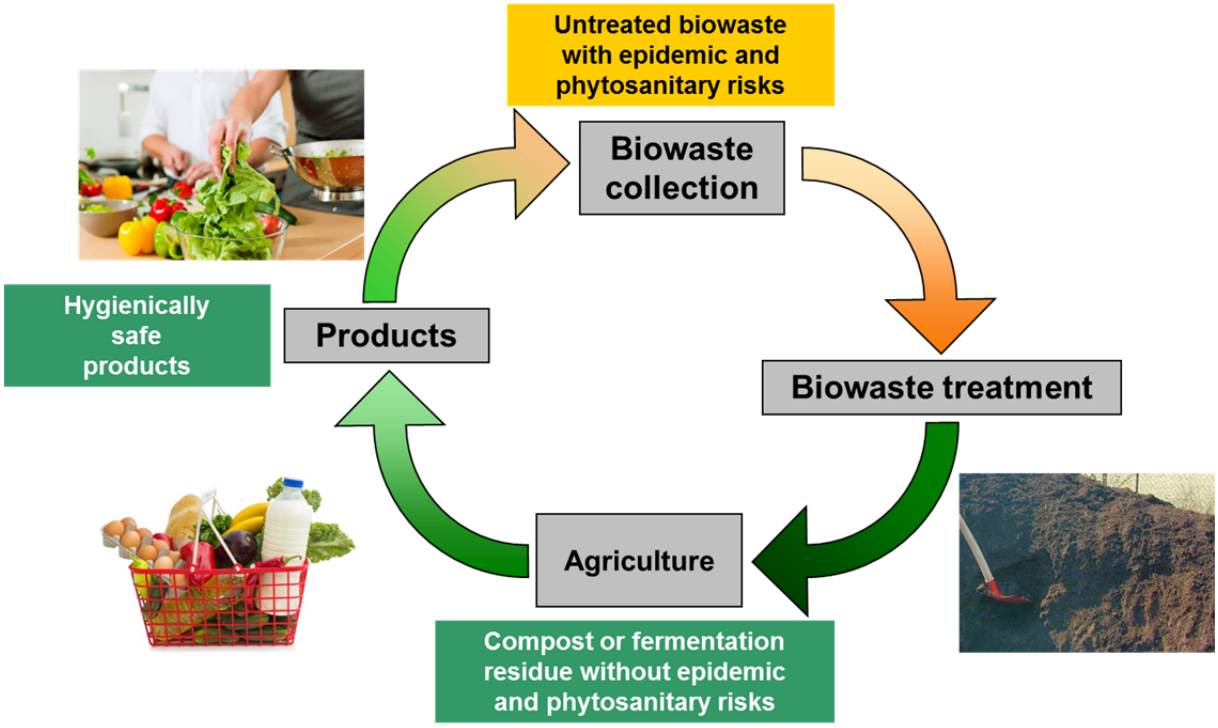


Figure 1: Hygiene status during biomass cycle

Residual waste, biowaste, packaging waste as well as waste paper and glass already have very high levels of bacteria and fungi on delivery to waste treatment plants, including diverse human-

[1] Hoppenheidt, K. (2012): Hygieneaspekte bei der getrennten Erfassung und Behandlung von Bioabfällen (Hygiene aspects of separate collection and treatment of biowaste). 6th Biomasseforum, 21/22 November 2012, Bad Hersfeld

pathogenic microorganisms[2]. People who work in the waste industry are given workplace-related instructions informing them about hygiene risks and suitable protective measures.

In private households residents are themselves responsible for compliance with hygiene precautions. However, data collated by the Robert Koch Institute (RKI) indicates significant gaps in knowledge, as private households are thought to be the starting point for more than 64 % of food-borne infections[3]. Accordingly, hygiene optimisation effects could be achievable above all in the way that private persons handle waste.

However, it was unknown whether the waste produced in households develops correspondingly high (microbial) contamination with bacteria and fungi after storage periods lasting a few days. In this case, waste would already be hygienically risky at the time of its collection in living spaces. Hygiene precautions - analogous to the health and safety measures to be taken in waste industry workplaces - should then be used when handling waste in the home. Primarily, direct skin contact with waste and inhalation of aerosols should be avoided.

This issue was taken up on behalf of BASF SE in an investigation programme using the example of household collection of biowaste. First it was examined by how much bacteria and fungi multiply in fresh biowaste during a storage period of up to 5 days at temperatures of 25 °C. Comparable conditions exist in households during summer weather conditions. Apart from germ growth in biowaste, the microbial contamination of surfaces, with which users can come into contact when handling biowaste, were also investigated. Here the collection of biowaste without waste bags was compared to collection in paper, PE and ecovio bioplastic bags (2.1). In addition, it was also examined whether differences result between collection of biowaste without bags and in ecovio bioplastic bags with regard to airborne bioburden on throwing the biowaste collected in households in the municipal biobins (2.2).

2 Investigation method and results

2.1 Hygiene status of biowaste collection in households

2.1.1 Preliminary remarks on the investigation framework

In moist biowaste produced in households, bacteria and fungi multiply particularly rapidly. The extent of microbial contamination of the waste collected in households is decisively dependent on the following influencing factors:

- Initial microbial contamination of the biowaste
- Time spent in the biowaste container
- Storage temperature
- Water content of the biowaste

Therefore, the following preliminary considerations were used to draw up the concept for the tests: In households around 81 kg biowaste per inhabitant and year are produced or rather around 220 grams per person. In Bavaria, 51 % of all 6.1 million private households are 1 and 2 person households; statis-

[2] Krist, H.; Hoppenheidt, K.; Mücke, W. (2005): Hygiene der Abfallentsorgung im Gesundheitswesen (Hygiene of waste management in the health care sector). Munich

[3] Bernard, H.; Stark, K. (2011): Lebensmittelbedingte Infektionen und Ausbrüche in Deutschland (Food-borne infections and outbreaks in Germany), RKI, Presentation, Rostock, 21.9.2011; www.lallf.de/fileadmin/media/PDF/Veroeffentlichungen/Veranstaltungen/BELA3.pdf

tically the average household size is 2.1 persons per household. With this data, an average biowaste production of approx. 460 grams per household and day was deduced for Bavaria. A bulk density of 0.7 kg/L or rather a specific volume of 1.4 L/kg was assumed for untreated biowaste. Accordingly around 0.65 litres of biowaste should be produced daily in an average household. Vessels frequently used for the collection of biowaste in households have maximum capacities of 5-10 litres. Therefore, the maximum fill capacity of the vessels would be reached after 10 days at the latest. If uniform daily biowaste production is assumed, the average dwell time of the biowaste is 5 days for use of a container volume for up to 10 days. The waste placed in the container last would only be stored in the waste container for one day; fractions of 10 % would be present in the waste container for up to 10 days. Even though in practice it is to be expected that the vessel is often emptied before the maximum capacity is reached, the estimation provided helpful framework data for drawing up the concept for the tests to be performed. Therefore, up to 5 days in 7 litre household collection containers was assumed as the time spent by the biowaste in the households and germ growth over this period was observed.

A defined biowaste mix was used to create initial conditions that were as comparable as possible. While many foods are stored chilled, biowaste is stored at comparatively high temperatures. A minimum temperature of 20 °C can be assumed all-year round in kitchens. Significantly higher temperatures can occur near heat sources (lighting, household appliances). In built-in kitchen units, waste bins are often located next to dishwashers or cookers, so that temperatures occur, at least for several hours, which are significantly higher than the room temperature. In the summer the room temperature can be well above the 20 °C mark for long periods during the day. Following the worst case approach, germ development at 25 °C was tested. At this temperature the bacteria and fungi occurring in environmental samples multiply very quickly.

Household biowaste usually has an average water content of over 70 %. However, individual biowaste constituents can have highly different water contents: Tomatoes, grapes and similar products have water contents higher than 95 % while, for example, the water content of bread is < 40 %. When making the investigated biowaste mixture it should be ensured that the average water content was > 70 %.

The initial microbial contamination of food waste can be scattered over a wide range. Foods frequently spoil easily; therefore attempts are made to extend their shelf life by preserving and/or chilling them. The waste of preserved and cooked foods starts off with lower initial germ levels. However, the waste of cooked foods become germ-ridden quickly, as cooking initiates the decomposition of many food constituents. Waste of untreated foods stored in cool or chilled conditions have high, natural germ levels (e.g. the colonisers of the surface of lettuce leaves or the germs in the earth adhering to potatoes, carrots, etc.). Microbially spoilt foods often also get into biowaste containers (e.g. mouldy bread, rotten fruit and vegetables). New containers and still edible biowaste constituents were used for the current study. Increased basic contamination is to be assumed for containers used for longer periods in households. In addition, in households, overstored, already spoilt food is also included in the biowaste collection. In order to reconstruct this increased initial microbial contamination in the study, several biowaste mixtures were inoculated with municipal biowaste. To this end, 20 grams of freshly delivered municipal biowaste (composting plant in Augsburg) was suspended in 200 mL ultrapure water. 25 mL of the suspension was then added to form a mixture with 2.7 kg freshly made biowaste. In this way the

biowaste made was enriched with one part per thousand of the microorganisms that exist in municipal biowaste.[4]

2.1.2 Microbiological investigation parameters

The hygienic tests included determination of the viable counts of bacteria and fungi (Table 1); parts of the samples were deep-frozen to preserve them for supplementary tests.

Table 1: Hygienic investigation parameters

Parameter	Medium, culture conditions	Area of application
Viable count of bacteria	CASO agar plates (with 50 mg/L nystatin and 25 mg/L actidione additive), 37 °C	Detection of sophisticated heterotrophic bacteria (TRBA 9430)
Viable count of moulds	DG-18 agar plates (with 100 mg/L chloramphenicol additive), 25 °C	Detection of xerophilic moulds (TRBA 9420)

2.1.3 Production of defined biowaste

A mixture of the components listed in Table 2 was made to fill the 7 L biowaste collection vessels.

Table 2: Components used to make defined biowaste

Constituent	Weighed portion in g/mix	Origin and preparation
Lettuce	550	Aldi, iceberg lettuce, preshredded
White cabbage	275	Fegro, white cabbage, preshredded
Tomatoes	275	Aldi, tomatoes, preshredded
Cucumbers	275	Aldi, cucumbers, preshredded
Potatoes (raw)	275	Fegro, potatoes, preshredded
Potatoes (cooked)	275	Fegro, potatoes, preshredded
Soybean meal	275	Baywa; Sojagold brand; unchanged
Coarse-ground wholegrain	140	Farmer; mixture of wheat and barley
Sweetcorn	275	Fegro, preserved sweetcorn, liquid removed
Cooked meat	140	Fegro, "JA" dog food (poultry with rice)
Total	2,755	

The preshredded to < 5 cm, loosely heaped biowaste had a stored density of 0.4 kg/litre. At the time it was placed in the biocontainer the defined biowaste had a water content of 76.7 %; the organic substance content DOM (dry organic matter) was 94.6 %. These values lie within a typical range of values for biowaste produced in households[5].

[4] During the course of the investigations it was found that the initial germ count of the biowaste was only slightly changed by the inoculation. Therefore, the biowaste was no longer additionally inoculated for further investigations (2.2).

[5] The waste delivered to biowaste treatment plants contain larger fractions of green (garden) waste; these contain woody constituents as well as soil and sand adhesions. Accordingly municipal biowaste has lower water contents (60 – 70 %) and smaller fractions of dry organic matter (60 – 80 %).

2.1.4 Biowaste collection containers and biowaste bags

The new vessels shown in Figure 2 were used as the collection containers. The plastic box (Fegro) was used without collection bags; as the lid was not perforated, it was only loosely placed on the box to ensure air exchange after the biowaste had been placed in it.

Figure 2: Waste container and biowaste bag variants used

The biowaste collection containers with ventilation slots (Stelo - biowaste bin; Biomasse GmbH) were fitted with one of the biowaste bags named in the following:

- Paper bag: dm Profissimo bio compost bag 10 L
- PE bag: 10 L bag, blackened[6]
- ecovio bag: 10 L biobag used in Bad Dürkheim

2.1.5 Viable counts of biowaste

The freshly made biowaste was examined for viable bacteria and fungi before and after inoculation with biowaste eluate. The biowaste placed in the various biowaste containers was stored for 5 days at 25 °C. The contents of the waste containers or biowaste bags were then examined for their levels of viable bacteria and fungi. The biowaste samples were emptied into clean plastic buckets disinfected by heating them to 70 °C beforehand. 2 litres of sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0) was then added. The bins were fitted with a tightly closing lid and incubated for 60 minutes at 25 °C on a horizontal shaker with 70 revolutions per minute.

[6] Tests on the BASF SE showed that the PE bag was 5-6 µm thick and was made of recycled plastic. Inclusions of small foreign particles can increase the permeability of recycled plastics. By contrast, the ecovio bags are approx. 25 µm thick and are made from a new, homogeneous polymer mixture.

The photos collated in Figure 3 of the fresh and 5 day old biowaste stored at 25 °C clearly show the visually identifiable changes: After 5 days the biowaste was more highly compacted, as individual biowaste constituents had lost their structure. Apart from the intensive odour, the already marked formation of a fungal mycelium on the surface of the biowaste was also conspicuous.

Without bag	Paper bag	PE bag	ecovio bag

Intensive odour, minor mould growth	Intensive odour, very extensive mould growth	Intensive odour; extensive mould growth	Intensive odour; extensive mould growth

Figure 3: Visual evaluation of the freshly added biowaste (top) and biowaste stored for 5 days at 25 °C

The eluates of the biowastes were diluted in suspension series with up to 9 decimal levels and from each suspension, 2 nutrient agar dishes each of the two nutrient media listed in Table 1 were inoculated. Reserved samples of the waste eluates were temporarily stored at -70 °C.

Following completion of the incubation, culture dishes with evaluable colony counts (limiting dilutions, cf. Figure 4) were selected and the numbers of colony forming units (CFU) were determined. Taking into account the weighed portions of biowaste and the volume of elution media, the numbers of colony forming units per gram biowaste fresh mass were determined.

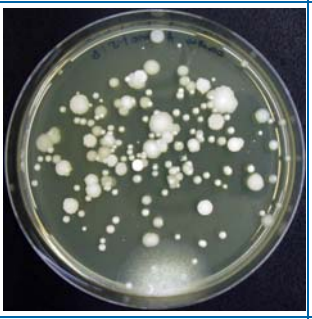
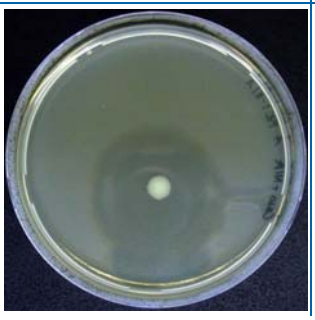
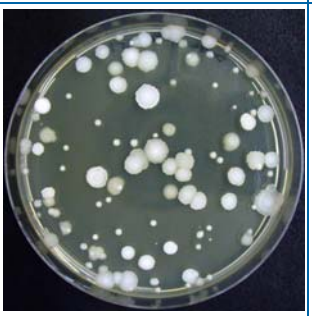
Fresh biowaste, uninoculated, 0 d		Fresh biowaste, inoculated, 0 d	
CASO, 10^{-2} dilution	DG 18, 10^{-1} dilution	CASO, 10^{-3} dilution	DG 18, 10^{-2} dilution
			
Biowaste without bag after 5 d at 25 °C		**Biowaste in paper bag after 5 d at 25 °C**	
CASO, 10^{-7} dilution	DG 18, 10^{-5} dilution	CASO, 10^{-8} dilution	DG 18, 10^{-5} dilution
			
Biowaste in PE bag after 5 d at 25 °C		**Biowaste in ecovio bag after 5 d at 25 °C**	
CASO, 10^{-8} dilution	DG 18, 10^{-5} dilution	CASO, 10^{-8} dilution	DG 18, 10^{-5} dilution
			

Figure 4: Colony patterns on limiting dilutions with evaluable colony counts

Note: Plates with few colonies were the only ones that were not fully overgrown.

The colony patterns show that a broad spectrum of bacteria, yeasts and fungi were present in the biowaste. The biowaste stored for 5 d at 25 °C had to be more highly diluted due to the massively increased germ counts by a factor of 1,000 (fungi) and by a factor of 10,000 to 100,000, before evaluable, individually formed colonies of bacteria and fungi existed. At the same time, it is conspicuous that the yeasts were more frequently found than molds on the DG 18 media. The high contents of biologically readily usable constituents in fresh biowaste apparently promote especially the growth of bacteria and yeasts. In tests on ripened composts, on the other hand, molds mostly dominate compared to yeasts.

Table 3: Results of the viable counts of bacteria (cfu) on CASO agar at 37 °C

Biowaste	CFU A per g solids	CFU B per g solids	CFU MW per g solids	CFU mean Total biowaste
Uninoculated, 0 d	5.8×10^4	8.3×10^4	**7.1×10^4**	**1.9×10^8**
Inoculated, 0 d	3.1×10^5	3.9×10^5	**3.5×10^5**	**9.6×10^8**
Without bag, 5 d	9.8×10^8	1.0×10^9	**9.9×10^8**	**2.5×10^{12}**
Paper bag, 5 d	1.3×10^9	9.5×10^8	**1.1×10^9**	**2.8×10^{12}**
PE bag, 5 d	3.7×10^8	5.9×10^8	**4.8×10^8**	**1.2×10^{12}**
ecovio bag, 5 d	6.5×10^8	4.9×10^8	**5.7×10^8**	**1.4×10^{12}**

Table 4: Results of the viable counts of fungi (cfu) on DG 18 agar at 25 °C

Biowaste	CFU A per g solids	CFU B per g solids	CFU MW per g solids	CFU mean Total biowaste
Uninoculated, 0 d	7.4×10^3	6.9×10^3	**7.2×10^3**	**2.0×10^7**
Inoculated, 0 d	3.4×10^4	4.0×10^4	**3.7×10^4**	**1.0×10^8**
Without bag, 5 d	1.3×10^8	1.6×10^8	**1.4×10^8**	**3.6×10^{11}**
Paper bag, 5 d	1.0×10^8	8.1×10^7	**9.1×10^7**	**2.3×10^{11}**
PE bag, 5 d	8.5×10^7	8.1×10^7	**8.3×10^7**	**2.1×10^{11}**
ecovio bag, 5 d	1.2×10^8	7.6×10^7	**9.6×10^7**	**2.4×10^{11}**

The results of the quantitative evaluations for the biowaste tested are summarised in Table 3 and Table 4. Figure 5 shows the mean values in graphic form. The freshly made biowastes contained 7.1×10^4 colony forming units (CFU) of bacteria and 7.2×10^3 CFU of fungi per gram of biowaste. The inoculation increased the initial levels by a factor of 5 only.

The biowaste stored for 5 d at 25 °C contained 5.7×10^8 – 1.1×10^9 CFU of bacteria and 8.3×10^7 – 1.4×10^8 CFU fungi per gram. These results substantiate the initial hypothesis that biowaste develops highly increased, hygienically risky germ levels during the few days of storage in the household.

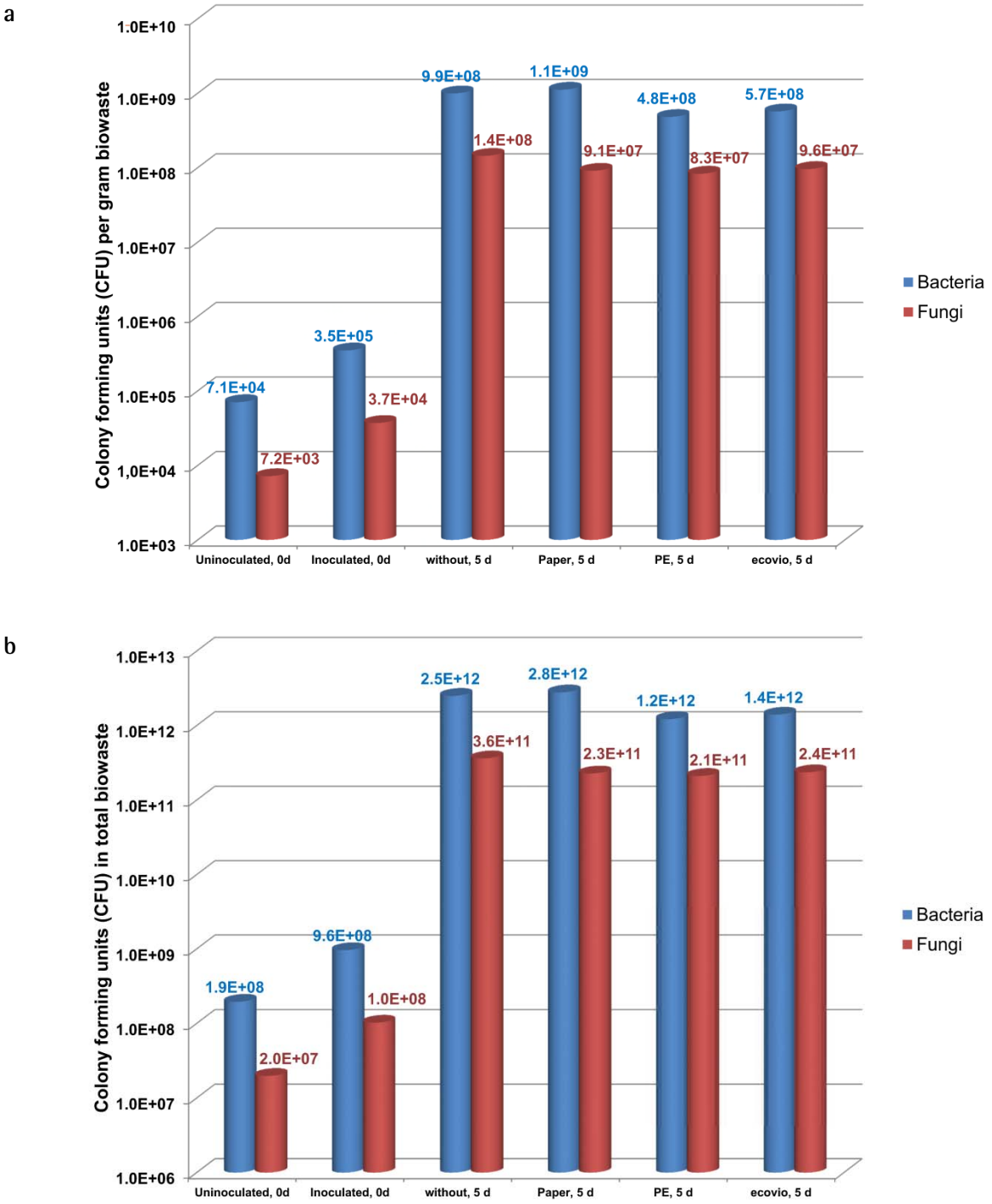


Figure 5: Results of the viable count determinations based on one gram of biowaste (a) and the total biowaste (b)

The total number of viable bacteria and fungi present in the contents of the filled biowaste containers were also calculated. The data was primarily used to evaluate those germ counts, which were determined on the respective contact surfaces of the biowaste containers or biowaste bags (see 2.1.6). Relative to the numbers of germs initially present in the inoculated fresh biowaste, the biowaste stored for

5 d at 25 °C contained numbers of bacteria increased by a factor of 1,268 to 2,902 bacteria and fungi numbers increased by a factor of 2,065 to 3,552.

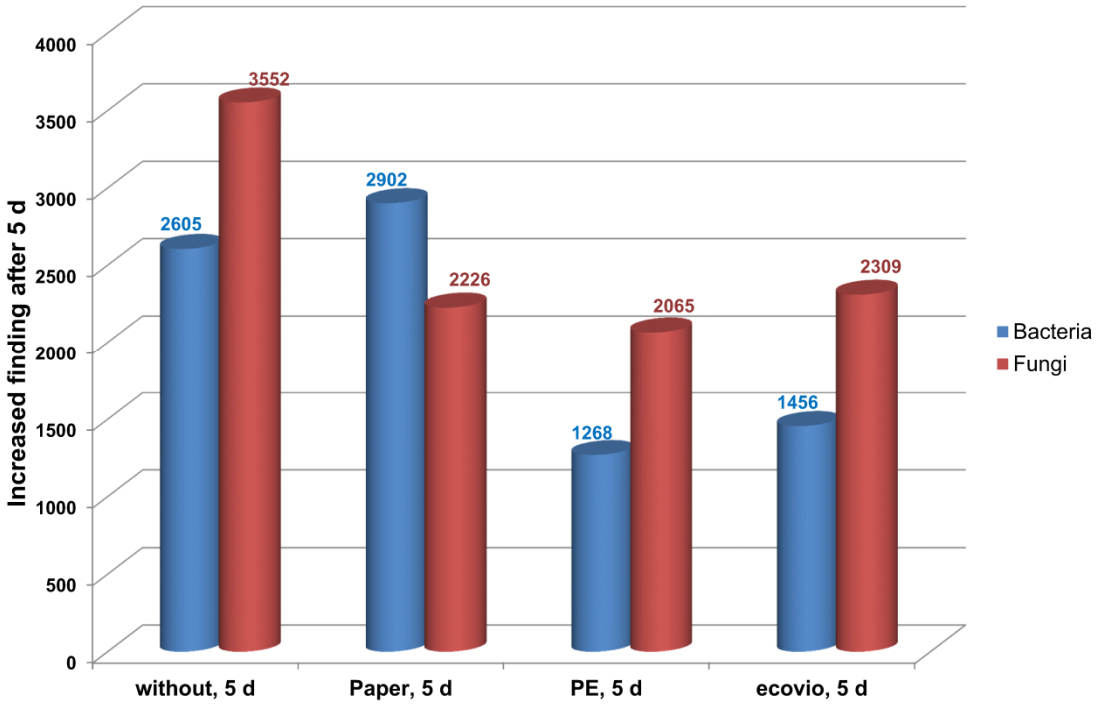


Figure 6: Increased findings of the viable counts of biowaste stored for 5 days at 25 °C compared to the initial value (inoculated biowaste); (calculated from data for the respective total waste quantities)

2.1.6 Viable counts of surfaces with biowaste contact

New biowaste bags and a new waste box, in which biowaste is to be stored without bags, were tested with regard to their initial germ levels. 100 mL sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0) was placed in the waste box; the box was closed and all internal surfaces were wetted by intensive shaking. The opening of the biowaste bags was folded several times and the fold was then stapled. The bags were placed in new PP plastic bags (autoclave bags) with 100 mL sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0). The PP plastic bag was closed and shaken thoroughly so that the surfaces of the bag to be tested were wetted and any germs present were carried into the elution liquid. The eluates were used for viable count determinations with the nutrient media listed in Table 1.

In order to be able to register lower initial levels too, 10 mL of the eluates were separated out on sterile membrane filters and the filters were then placed on the agar surfaces. Despite this large enrichment, a maximum of 1 or 2 colony forming units of bacteria were found on the filters. Filters placed on DG 18 agar remained without growth. Therefore, an initial microbial contamination of < 15 colony forming units was deduced relative to the respective total surface[7] for the tested surfaces of the waste box and the biowaste bags. Therefore, mathematically, there were less than 0.003 to 0.006 CFU/cm² of bacteria and fungi present.

Figure 7 to Figure 11 contain the photo documentation of the biowaste containers stored for 5 d at 25 °C. The summaries also contain supplementary notes on particular observations.

[7] Waste box: ~ 2,500 cm²; paper bag: ~ 2,500 cm²; PE bag: ~ 5,400 cm²; ecovio bag: ~ 4,000 cm²

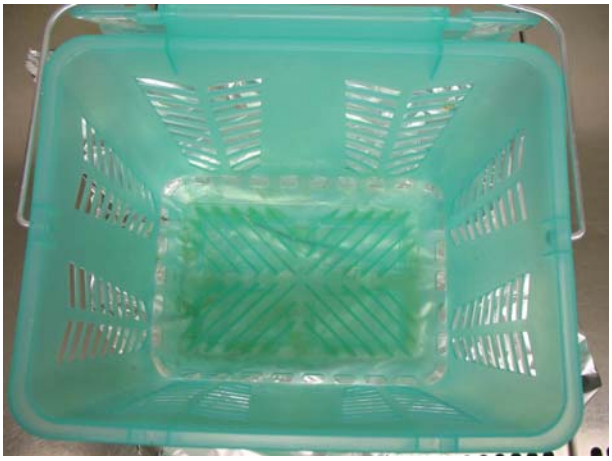
Intensive odour.

Within 5 days the biowaste had significantly lost its structure and was clearly compacted. In the bottom/floor area it has an almost pulpy / paste-like consistency. Localised molds were visible on the surface.

After emptying small quantities of biowaste remained on the inner surface. Before the next use it was therefore necessary to clean the inside surfaces.

Figure 7: Photo documentation of the biowaste stored without bags for 5 d at 25 °C

After the biowaste was emptied out this waste box used without bags had intensive smelling dirt on its inside surfaces. As this dirt had to be removed before further use, the inside surface was classified as being a possible contact surface. By analogy to the testing of the initial microbial contamination, 100 mL sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0) was therefore added to the waste box. The closed box was placed in a PP plastic bag to protect against leaking splashing fluid. The box was shaken intensively for 60 minutes on a horizontal shaker to elute the germs adhering to the inside of the waste box (see Figure 12).



Intensive odour.

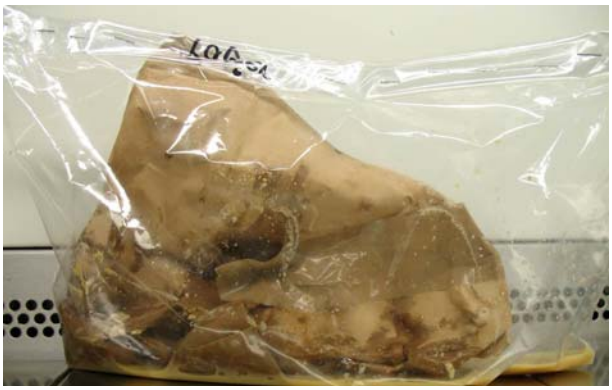
Very extensive fungal infestation on the surface; liquid discharge in the bottom area; bottom of the paper bags with growth on the outside and paper completely softened; biowaste escaped as soon as the bag was lifted.

Cracks were covered with adhesive parcel tape for elution of the contact surfaces, yet the paper dissolved in some places.

Handling more problematic than for collection without bags.

Figure 8: Photo documentation of the biowaste stored in paper bags for 5 d at 25 °C

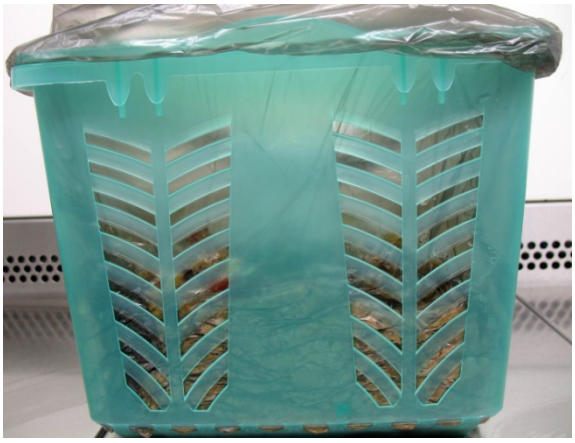
The paper bag filled with waste was not discernibly wet through on the side surfaces after storage for 5 d at 25 °C. However, when the bag was lifted massive microbial growth was discovered on the outside of the bottom of the bag. In addition, brownish discoloured liquid had escaped and had contaminated the bottom of the waste container. During a preliminary test a paper bag was still structurally stable after 10 days of storage in pure water (Figure 9). However, the paper bag filled with biowaste was already very unstable in the bottom area of the bag. On emptying out the biowaste the bottom area of the bag tore and had to be stabilised with adhesive tape for the subsequent elution. In analogy to the procedure for determination of the initial microbial contamination, the bag opening was again folded several times and the fold was stapled. The bag was then placed in a new PP plastic bag with 100 mL sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0). The sealed PP bag was then shaken for 60 minutes on a horizontal shaker.

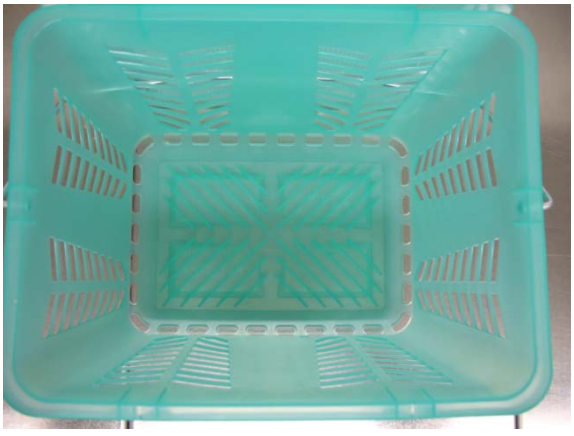


The wet stability of the biowaste collection bag made of paper had been checked in a preliminary test. Following storage for 10 days in tap water it was softened but still intact.

The bag filled with biowaste for 5 days was softened and unstable in the area at the bottom of the bag. The surface was covered with a white coating made of microorganisms. Accordingly the eluate of the outer surface of the bag was extremely cloudy (turbid).

Figure 9: Photo of a paper biowaste bag stored for 10 days in tap water (left) and a paper biowaste bag filled with biowaste and stored for 5 days (right)





Intensive odour.

Extensive fungal growth on the surface. Bag leaktight and outside dry. The collection container was unsoiled.

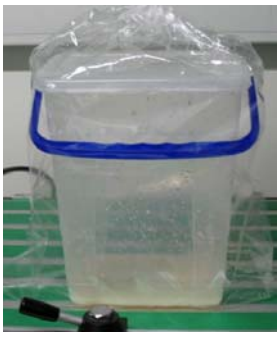
Figure 10: Photo documentation of the biowaste stored in PE bags for 5 d at 25 °C

Intensive odour.

Extensive fungal growth on the surface. Bag leak-tight and outside dry. The collection container was unsoiled.

Figure 11: Photo documentation of the biowaste stored in ecovio bags for 5 d at 25 °C

After 5 d at 25 °C the PE bag and the ecovio bag were unsoiled and dry on their outer surfaces. There was no leaked biowaste liquid in the waste collection vessel. In analogy to the determination of the initial microbial contamination the openings of the bag were folded after the bag had been emptied and the fold was stapled. The biowaste bag was then placed in a new PP bag with 100 mL sterile, 0.01 molar sodium pyrophosphate solution (pH 7.0). The sealed PP bag was then shaken for 60 minutes on a horizontal shaker.



Elution bags: Bags were sealed and placed together with 100 mL elution medium in new PP bags and shaken horizontally for 60 minutes.
Elution box: Elution – medium was placed in the box and the inner contact surfaces were eluated

Figure 12: Photo documentation of the surface elution

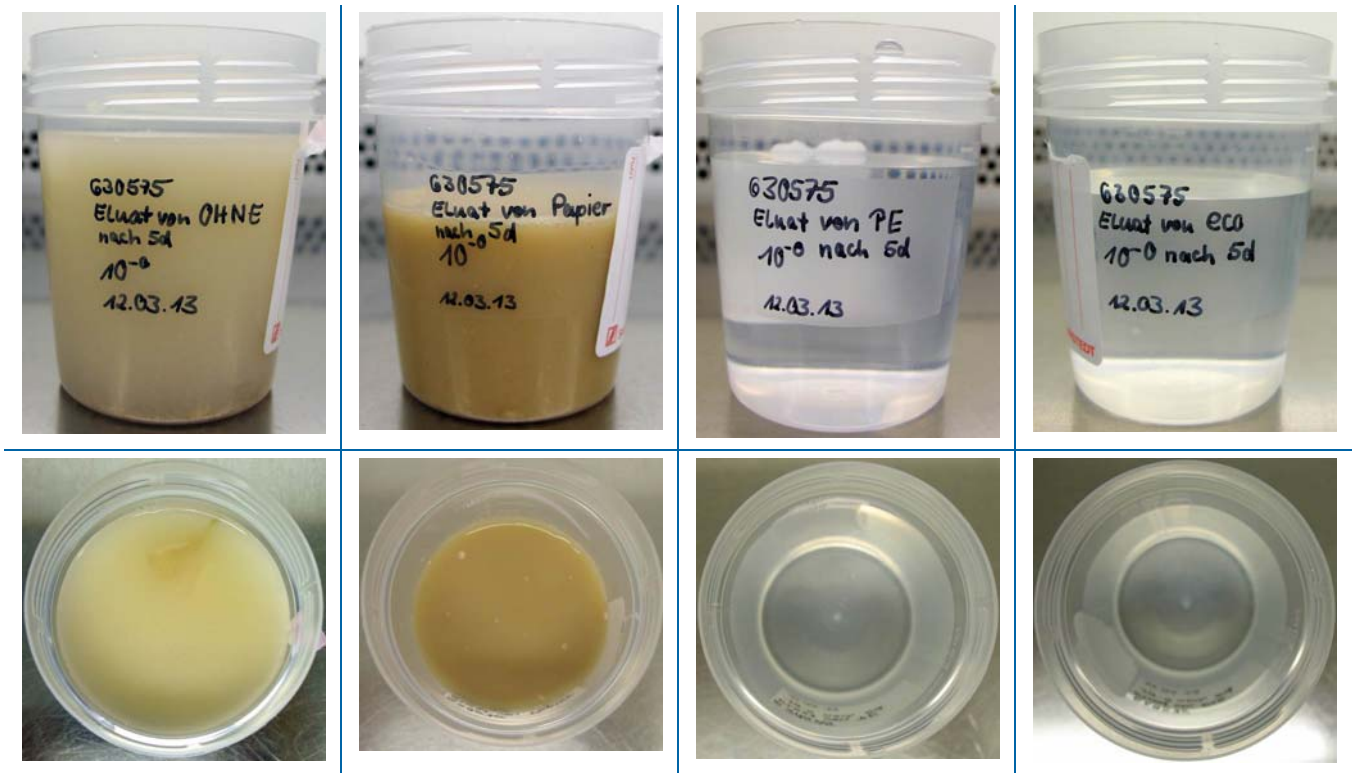


Figure 13: Photo documentation of the contact surface eluates

The eluates of the inside surface of the waste box and the outside surface of the paper bag were intensively cloudy and had yellowish-brownish discolouration. The eluates of the outside surfaces of the PE bag and the ecovio bag were not cloudy or turbid and were not discoloured (Figure 13).

Table 5: Viable counts of bacteria (cfu) for the eluates of the tested surfaces

Biowaste	CFU A in the eluate	CFU B in the eluate	CFU mean in the eluate	CFU mean* per cm²
Without bag, 5 d	9.8×10^9	8.9×10^9	**9.3×10^9**	**3.7×10^6**
Paper bag, 5 d	2.1×10^{11}	4.0×10^{11}	**3.1×10^{11}**	**1.2×10^8**
PE bag, 5 d	1.3×10^7	1.3×10^7	**1.3×10^7**	**2.4×10^3**
ecovio bag, 5 d	1.1×10^6	6.9×10^5	**8.8×10^5**	**2.2×10^2**

* calculated from the germ counts of the eluates and the eluated surfaces

Table 6: Viable counts of fungi (cfu) for the eluates of the tested surfaces

Biowaste	CFU A in the eluate	CFU B in the eluate	CFU mean in the eluate	CFU mean* per cm²
Without bag, 5 d	2.5×10^9	2.9×10^9	**2.7×10^9**	**1.1×10^6**
Paper bag, 5 d	2.5×10^{10}	2.4×10^{10}	**2.4×10^{10}**	**9.7×10^6**
PE bag, 5 d	7.3×10^5	8.8×10^5	**8.0×10^5**	**1.5×10^2**
ecovio bag, 5 d	1.1×10^5	1.0×10^5	**1.0×10^5**	**2.6×10^1**

* calculated from the germ counts of the eluates and the eluated surfaces

In line with the visual impression, the determinations of the viable counts showed highly increased findings for the eluates of the mixture without bag and the mixture with paper bag (Table 5, Table 6, Figure 14).

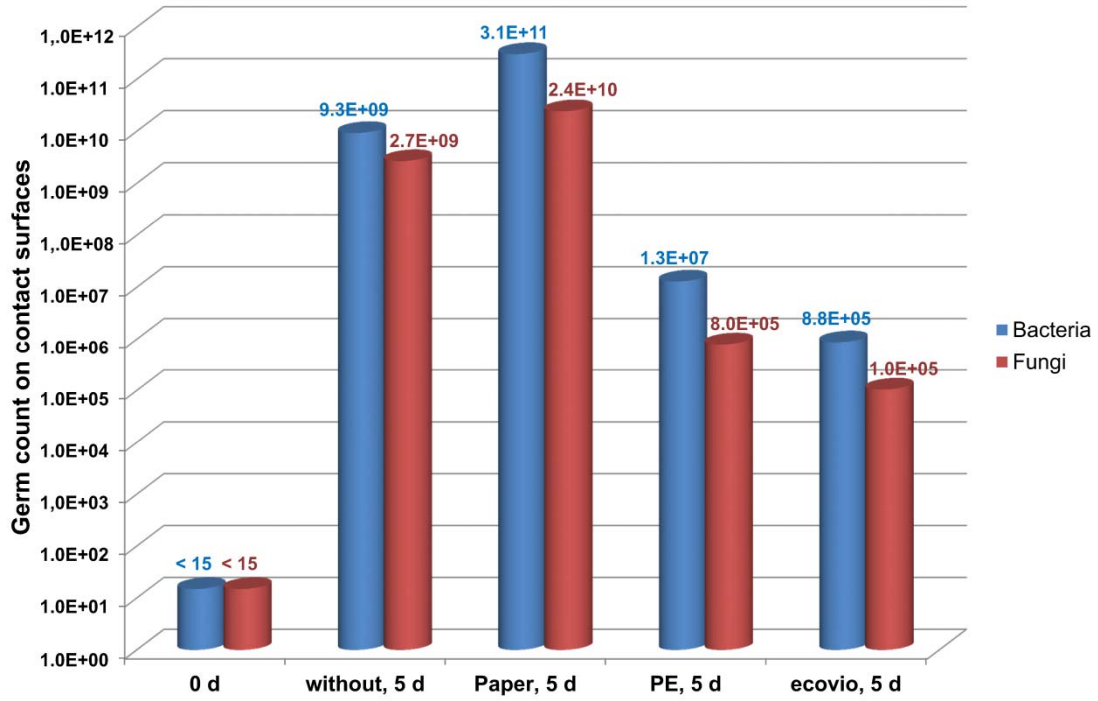


Figure 14: Results of the viable count determinations for the eluates of the contact surfaces

Before use the tested surfaces had viable counts below the detection limit of 15 colony forming units (CFU). After use 9.3 x 10^9 CFU of bacteria and 2.7 x 10^9 CFU of fungi were measured on the inside surface of the waste box without bag. Therefore, its use increased microbial contamination by more than a factor of 6.2 x 10^8 (bacteria) and 1.8 x 10^8 (fungi). Based on one square centimetre of the tested surface, 3.7 x 10^6 CFU of bacteria and 1.1 x 10^6 CFU of fungi were found.

In the case of the paper bag, even higher microbial contamination was found on the surfaces following use: In total, 3.1 x 10^{11} CFU bacteria and 2.4 x 10^{10} CFU fungi were found. The use therefore increased microbial contamination by more than a factor of 2.0 x 10^{10} (bacteria) and 1.6 x 10^9 (fungi). Based on one square centimetre of the tested surface, 1.2 x 10^8 CFU of bacteria and 9.7 x 10^6 CFU of fungi were found. (Remark: The bottom of the bag was visibly covered in growth and its surface will have had a significantly higher colonisation density).

On the tested surfaces of the PE and ecovio bags only slightly increased levels of viable bacteria and fungi were found. After use, the surface of the PE bag had 1.3 x 10^7 CFU of bacteria and 8.0 x 10^5 CFU of fungi. The use therefore increased microbial contamination of the outside surface of the PE bag by more than a factor of 8.7 x 10^5 (bacteria) and 5.4 x 10^4 (fungi). However, on the surface of the PE bag only 2.4 x 10^3 CFU bacteria and 1.5 x 10^2 CFU fungi per cm^2 were measured. These values are lower than the germ count found on normal palms[8] of > $10^4/cm^2$ (Figure 15).

[8] Spradlin, C. T. (1980): Bacterial abundance on hands and its implications for clinical trials of surgical scrubs. In: Journal of Clinical Microbiology 11 (4), p. 389–393.

The surface of the ecovio bag had the lowest microbial burden after use: 8.8 x 10^5 CFU of bacteria and 1.0 x 10^5 CFU of fungi were measured. The use therefore increased microbial contamination of the outside surface of the ecovio bag by more than a factor of 5.9 x 10^4 (bacteria) and 7.0 x 10^3 (fungi). However, on the surface of the ecovio bag only 2.2 x 10^2 CFU bacteria and 2.6 x 10^1 CFU fungi per cm^2 were measured. These values are significantly lower than the germ count found on normal palms[8] of > $10^4/cm^2$ (Figure 15).

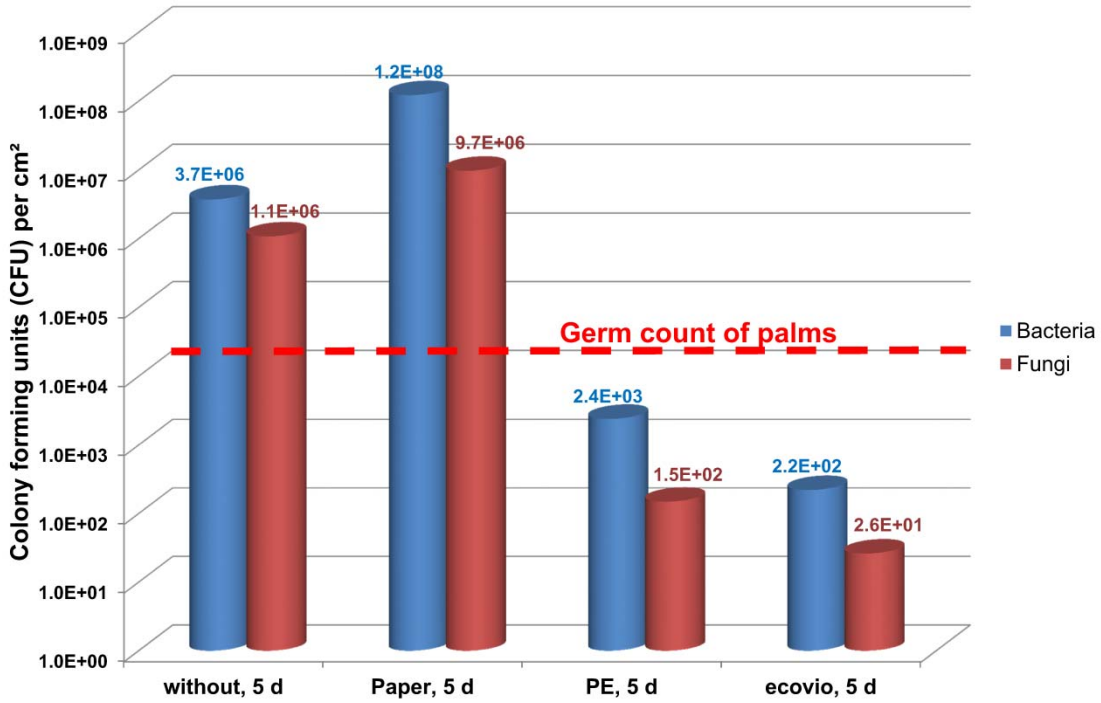


Figure 15: Results of the viable count determinations for contact surfaces

Another comparison clearly shows that use of adequately resistant bag materials can very highly limit contact with the high germ counts produced in the biowaste (Table 7). To this end, the germ counts of the tested contact surfaces were considered in relation to the germ counts of the biowaste stored for 5 days. The numbers of viable bacteria and fungi found on the PE bags represent only 0.0011 and 0.0004 % respectively of the bacteria and fungi that exist in the biowaste. The retention effect of the ecovio bag was even more marked: The numbers of viable bacteria and fungi found on the surface of the bags represent only 0.00006 and 0.00004 % respectively of the bacteria and fungi that exist in the biowaste.

Table 7: Proportion of the germs that exist on the contact surfaces in relation to the total germ levels of the biowaste

	Without bags	Paper bag	PE bag	ecovio bag
$VC_{Bacteria}$	0.37 %	10.9 %	0.0011 %	0.00006 %
VC_{Fungi}	0.74 %	10.7 %	0.0004 %	0.00004 %

2.2 Comparative investigation of the hygiene status of biowaste collection in biobins

2.2.1 Investigation method

In addition to the tests described in 2.1, it was also examined whether the type of biowaste collection in the household affects the airborne germ burden when throwing the biowaste into the municipal biowaste container. The collection variants examined were waste box without bag and biowaste collection with ecovio bag. Additional inoculation of the biowaste was not used, as very high germ levels were produced in the biowaste collection containers after the 7 day standing periods.

Over a period of 14 days, fresh biowaste (see Table 2) was produced on each working day and initially pre-incubated for 7 days in 2 household collection containers each at 25 °C, so that the typical initial microbial contamination of the waste was present.

Figure 16: Biowaste stored for 7 days at 25 °C in ecovio bags (left) and without bags

2 biowaste batches each collected in waste boxes without bag and 2 collected in ecovio bags and then pre-incubated were thrown into clean 120 litre biobins on each working day (Figure 17). The biobins were set up at 20 °C; this temperature lies within the average summer temperature in Germany.

Figure 17: Biowaste stored in ecovio bags (left) and without bags after being thrown into the biobins

Following a collection period of 7 and 14 days comparative measurements of the airborne germ burdens were taken when throwing in the biowaste bags.

Two airborne germ samples were taken at the same time in each sampling location. In total, the following sampling was carried out on both sampling days:

- **Background concentration** of the room air: 2 filters
- Biobin with biowaste in **ecovio bags before being thrown into the bin**: 2 filters
- Biobin with biowaste in **ecovio bags after being thrown into the bin**: 2 filters
- Biobin with biowaste **without bags before being thrown into the bin**: 2 filters
- Biobin with biowaste **without bags after being thrown into the bin**: 2 filters

The airborne germ collectors were positioned at the level of the bin lid. Before the biobins were opened the background values were measured. The biobin filled with ecovio bags was opened first and the airborne germ sampling was carried out immediately. The airborne germ level was therefore recorded without whirling up the air by throwing in bags. The two ecovio biowaste bags were then thrown in and the biobin was lifted by several centimetres and dropped several times with the lid closed. The turbulence was intended to create a situation found by a person who opens the biobin shortly after throwing in the first bag (or the biobin transport). The biobin lid was then opened and a renewed airborne germ sample was taken. The procedure was then repeated for the biobin with biowaste collected without bags.

Figure 18: Photo documentation of the airborne germ sampling

The characteristic data of the airborne germ collection is summarised in Figure 19. With the help of the Sartorius MD8 airborne germ collector, 1,000 litres of air were extracted in 10 minutes and the bioaerosol constituents present in the air were separated out on Sartorius gelatine filters.

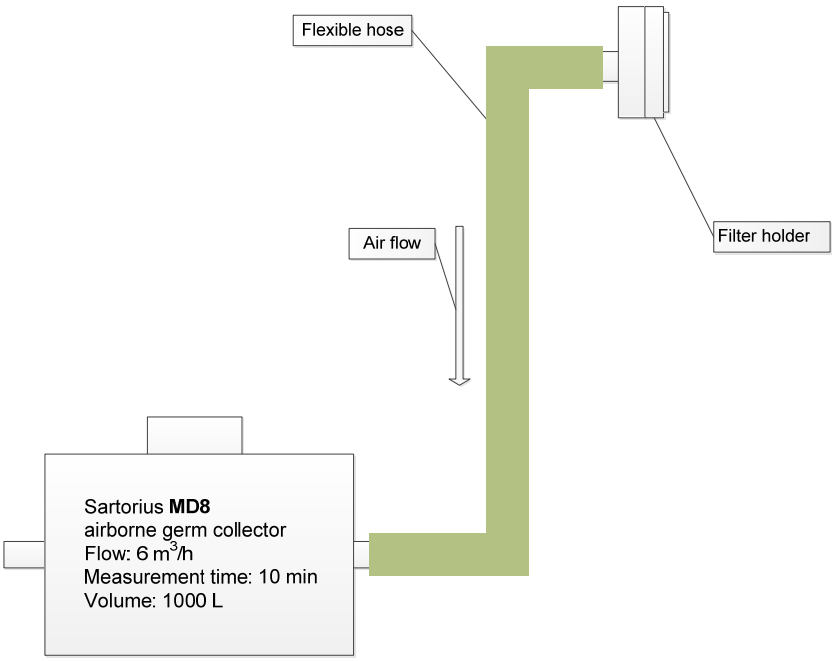


Figure 19: Characteristic data of the airborne germ collection

The filters were packed with 10 mL sterile NaCl solution and incubated in the water bath for 15 minutes at 37 °C. This caused the gelatine to dissolve completely and the germs were quantitatively released. A serial, decimal suspension series was created from the respective original suspension. 100 µL part from each dilution level was plated on 2 CASO agar plates using sterile Drigalski spatulas to determine the bacterial germ count and on 2 DG-18 agar plates to determine mesophilic fungi (see Table 1). The CASO plates were incubated at 37 °C for 24 h, the DG-18 plates at 25 °C for 72 - 120 h. As several samples were expected to have very low airborne germ levels, in addition 1 mL part of the filter eluates was placed on CASO and DG18 nutrient agar dishes and pre-dried under the safety workbench.

2.2.2 Investigation results

In total, 20 biowastes without bags and 20 biowastes in ecovio bags were thrown into the respective biobin. The biowaste pre-incubated for 7 days at 25 °C were already highly microbially colonised. Due to loss of structure the biowaste was significantly compacted and liquid had accumulated in the bottom area of the bin. In the case of four of the 20 biowaste collections in ecovio bags liquid escape from the bag was observed. The bottom area of the collection vessel was contaminated as a result. However, the ecovio bags were visually undamaged. The liquid apparently escaped through leaking seams or tiny holes in the film. In 16 of 20 biowaste collections the collection vessel used with ecovio bags was not soiled. On the other hand, all 20 waste boxes used without boxes were highly contaminated with adherent biowaste after they were emptied.

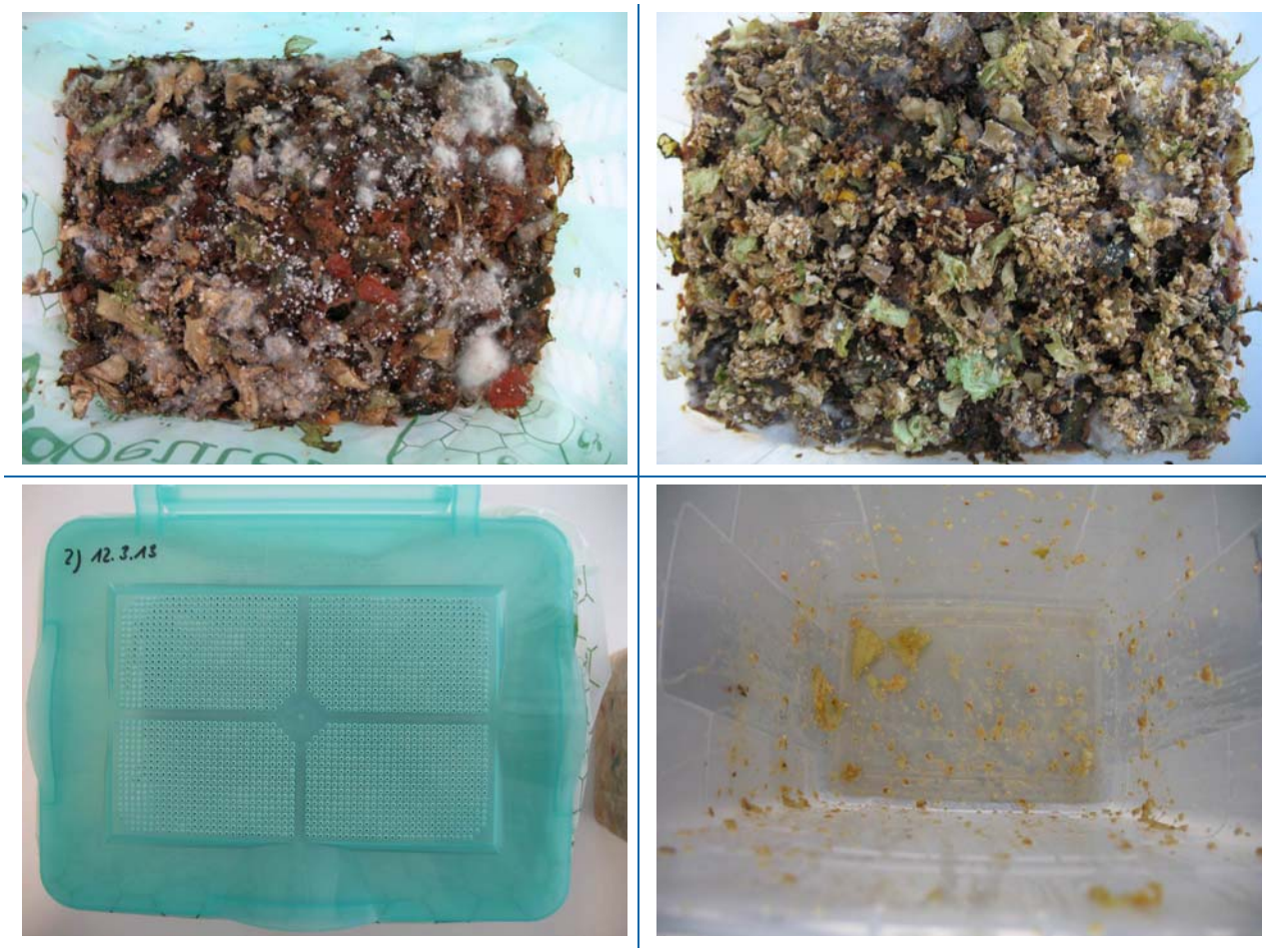


Figure 20: Photo documentation of the biowaste pre-incubated for 7 d at 25 °C and the emptied collection vessels

Table 8 and Table 9 show collations of photos of those culture dishes that were inoculated with one millimetre of the filter suspensions. The microorganisms separated out of 0.1 m³ air were inoculated on the culture dishes. The visual comparison shows that not only after a standing time of 7 days but also after 14 days, only very low airborne germ levels were determined before the biowaste was thrown into the bins. The values are not significantly different to the very low initial values (background). This finding is plausible: The biowaste was very moist and had an almost pasty consistency. This meant that there was little dust generation and associated increase in airborne germs.

Only after the biowaste was thrown in – each time with mechanical shaking of the biobin – were increased airborne germ levels detectable. The photos of the colony patterns and the quantitative evaluations in Table 10 show clearly that when the biowaste packed in the ecovio bags was thrown into the bin only a slight increase in airborne germs was observed. 578 and 238 CFU/m³ of bacteria and 210 and 150 CFU/m³ of fungi were detected respectively.

An evaluation of the Institute for Occupational Safety of the German statutory accident insurance (Deutsche Gesetzlichen Unfallversicherung - IFA) is available as a comparison basis. IFA evaluated and

published a larger database of levels of bacteria, fungi and endotoxins occurring in the outdoor air[9]. The outdoor air values for bacteria (mean: 285 CFU/m³; min./max.: 22 – 2,190 CFU/m³; measured values: 216) and endotoxins (mean: 6.7 EU/m³; min./max.: 0.3 - 77.7 EU/m³; measured values: 191) fluctuated only a little during the course of the year. The levels of fungi in outdoor air (mean: 1,584 CFU/m³; min./max.: 96 – 15,200 CFU/m³; measured values: 665) reached values in the months May to October which were mostly above 1,000 CFU/m³; during the cold seasons fewer fungi were observed in the air.

On this comparative basis, the airborne germ levels occurring when biowaste packed in ecovio bags are thrown into the bins can be allocated to typical outdoor air concentrations.

On the other hand, when the biowaste collected in the biobin without bags was thrown in the bin airborne germ levels classified as significantly increased occurred in comparison to typical outdoor air: 26,500 and 11,000 airborne bacteria and 6,500 and 30,250 airborne fungi were detected. These values are higher by a factor of 46 (bacteria) and by a factor of 31 – 202 (fungi) than the values observed when the biowaste packed in ecovio bags was thrown into the bin.

[9] Kolk, A.; Van Gelder, R.; Schneider, G.; Gabriel, S. (2009): Mikrobiologische Hintergrundwerte in der Außenluft – Auswertungen der BGIA-Expositionsdatenbank MEGA (Microbiological background levels in ambient air - evaluations of the IFA-MEGA exposure database). Gefahrstoffe - Reinhaltung der Luft, 69, 4, 130-136

Table 8: Colony patterns of the culture dishes inoculated with 1 mL filter suspension – airborne germ sampling after 7 day standing time of the biobins

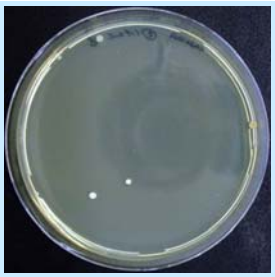
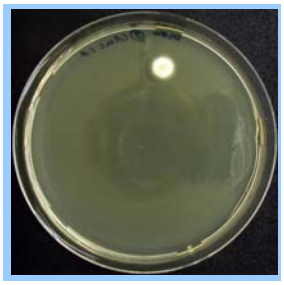
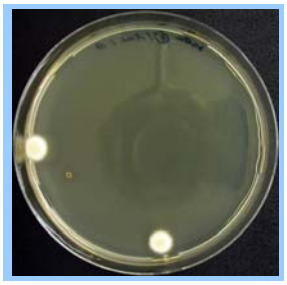
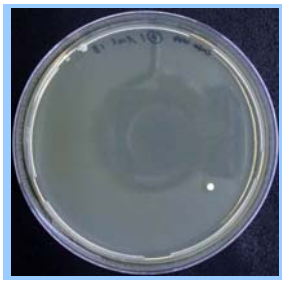
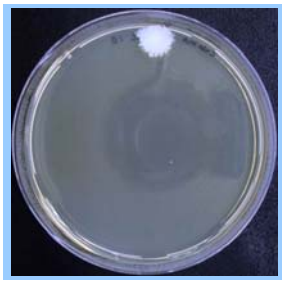
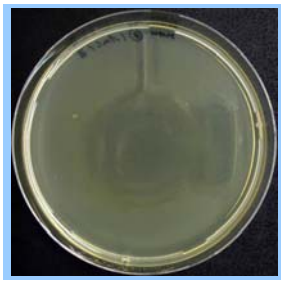
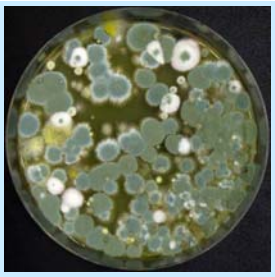
Sample	CFU bacteria		CFU fungi	
	Filter A	Filter B	Filter A	Filter B
Background				
ecovio before being thrown in				
ecovio after being thrown in				
Without bag before being thrown in				
Without bag after being thrown in				

Table 9: Colony patterns of the culture dishes inoculated with 1 mL filter suspension – airborne germ sampling after 14 day standing time of the biobins

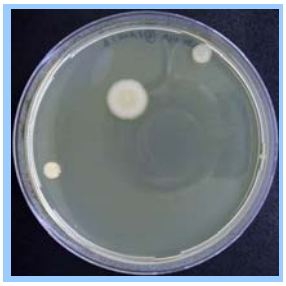
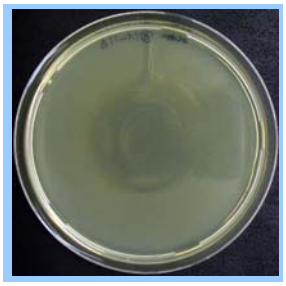
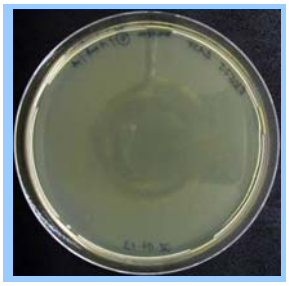
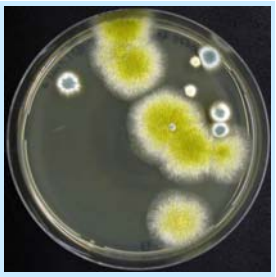
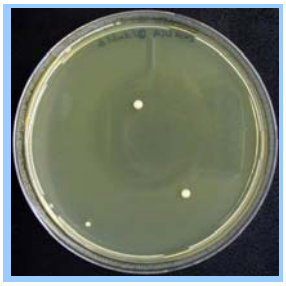
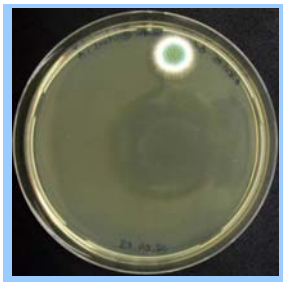
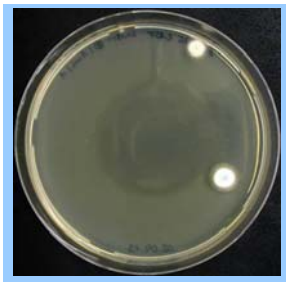
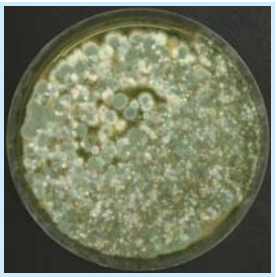
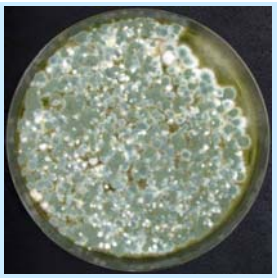
Sample	CFU bacteria		CFU fungi	
	Filter A	Filter B	Filter A	Filter B
Background				
ecovio before being thrown in				
ecovio after being thrown in				
Without bag before being thrown in				
Without bag after being thrown in				

Table 10: Concentrations of airborne bacteria and fungi

CFU/m³	CFU bacteria			CFU fungi		
Background	**Filter A**	**Filter B**	**Mean**	**Filter A**	**Filter B**	**Mean**
7 d	< 10	40	**< 25**	< 10	< 10	**< 10**
14 d	30	60	**45**	< 10	60	**< 35**
ecovio before being thrown in	**Filter A**	**Filter B**	**Mean**	**Filter A**	**Filter B**	**Mean**
7 d	< 25	< 10	**< 18**	< 10	20	**< 15**
14 d	35	25	**30**	< 10	< 10	**< 10**
ecovio after being thrown in	**Filter A**	**Filter B**	**Mean**	**Filter A**	**Filter B**	**Mean**
7 d	650	505	**578**	250	170	**210**
14 d	355	120	**238**	170	130	**150**
without bag before being thrown in	**Filter A**	**Filter B**	**Mean**	**Filter A**	**Filter B**	**Mean**
7 d	< 25	10	**< 18**	< 15	20	**< 18**
14 d	25	25	**25**	< 10	< 15	**< 13**
without bag after being thrown in	**Filter A**	**Filter B**	**Mean**	**Filter A**	**Filter B**	**Mean**
7 d	36,500	16,500	**26,500**	9,500	3,500	**6,500**
14 d	14,500	7,500	**11,000**	44,500	16,000	**30,250**

3 Conclusion

The investigation programme covered two hygiene issues that arise with household collection of biowaste. First it was examined by how much bacteria and fungi multiply in fresh biowaste during a storage period of up to 5 days at temperatures of 25 °C. Comparable conditions exist in households during summer weather conditions. Apart from germ growth in biowaste, the microbial contamination of surfaces, with which users can come into contact when handling biowaste, were also investigated. Here the collection of biowaste without waste bags was compared to collection in paper, PE and ecovio bioplastic bags. In addition, it was also examined whether differences result between collection of biowaste without bags and in ecovio bioplastic bags with regard to airborne bioburden on throwing the biowaste collected in households in the municipal biobins.

The bacteria (3.5×10^5/g) and fungi ($3,7 \times 10^4$/g) present in fresh biowaste multiplied very significantly within 5 days. The concentrations of bacteria (5.7×10^8/g to 1.1×10^9/g) and fungi (8.3×10^7/g to 1.4×10^8/g) reached orders of size (Figure 5), which are comparable with the levels of residual and biowaste classified as hygienically risky on delivery in waste recovery plants[10]. These results confirm that hygiene precautions should be taken when handling biowaste collected in households. This primarily includes avoiding direct skin contact.

If biowaste in the household is collected in containers without bags the soiled surfaces have to be cleaned. Very high microbial contaminations were found on the tested surfaces with 3.7×10^6 bacte-

[10] Krist, H.; Hoppenheidt, K.; Mücke W.: Hygiene der Abfallentsorgung im Gesundheitswesen. Munich, 2005

ria/cm² and 1.1 x 10^6 fungi/cm² (Figure 15). As many human-pathogenic germs can also multiply in biowaste, direct skin contact should be avoided for reasons of protection against infection.

Very high microbial contamination of the contact surfaces was also observed in the examined collection of biowaste in paper bags, with 1.2 x 10^8 bacteria/cm² and 9.7 x 10^6 fungi/cm². In the area of the bottom of the paper bag, leaking waste suspension had caused massive microbial growth to develop on the outside surface of the bag and had destabilised the bottom of the bag. As a result the hygiene risks when handling biowaste collected in paper bags was even more unfavourable than for collection without bags.

In the case of the biowaste collected in PE and ecovio bioplastic bags only very low microbial contaminations were detectable on the outer contact surfaces. 2.4 10^3 bacteria/cm² and 1,5 x 10^2 fungi/cm² were found on the outer surface of the PE bag. The microbial contamination of the outside surface of the ecovio bioplastic bag was lowest. 2,2 x 10^2 bacteria/cm² and 2.6 x 10^1 fungi/cm² were found. These values are significantly lower than the germ levels found on the skin of unsoiled palms of hands (> 10^4/cm²).

The different microbial contamination of the surfaces of the aids used to collect biowaste in households also affects the airborne germ burden when the biowaste is thrown into 120 L biobins. The airborne germ levels in the opening area of the biobins in which biowaste was collected over 7 and 14 days were compared. Biowaste collected without bags was emptied in one biobin. Biowaste collected in ecovio bioplastic bags was emptied into the second biobin.

Due to the high water contents of the biowaste only slight dust development was observed. Therefore, opening the biobins alone did not affect the airborne germ levels. Only after biowaste had been thrown into the bins and the biobins were shaken mechanically were increased airborne germ levels determined on renewed opening of the biobins.

The air of the biobin, in which biowaste collected in ecovio bioplastic bags had only slightly increased airborne germ levels: The values lay within the average range of the germ levels of normal outdoor air. The barrier function of the ecovio bioplastic bags prevented massive release of the bacteria and fungi enclosed in the bags, even after 14 day storage of the biowaste in the biobin.

By comparison, significantly increased levels of bacteria and fungi were found in the air of the biobin in which biowaste collected without bags was thrown, after the waste was thrown in and the bins shaken mechanically. The airborne germ levels were higher by a factor of 46 (bacteria) and by a factor of 31 – 202 (fungi) than the values observed when the biowaste packed in ecovio bags was thrown into the bin.

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