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# Microbial purity of recycled fibers made from printed offset paper and nanomodified polycaprolactone coated paperboard

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## Abstract

In order to increase the sustainability of paper or board production, it is desirable to use recycled fibers as much as possible. Microorganisms are in a smaller or higher amount present on the surface of paper or paperboard, so they are also present in the paper pulp or on the cellulose fibers. The purity of the mentioned fibers is important for obtaining a quality raw material that is health conforming. The aim of this study is to determine the microbiological quality of recycled fibers obtained by recycling of paper and paperboard intended for the manufacture of packaging products. Samples were in an average microbiological environment without food exposure. Quality of recycled fibers was studied through the total number of bacteria and determined for different recycled samples. The total number of microorganisms was estimated by both the disintegration and smear method. Results showed that only the disintegration method was suitable for the evaluation since the smear method did not produce any results. Moreover, the disintegration method was suitable only for the determination of bacteria alone, since no growth of molds or yeast occurred. In addition, the influence of paper composition, paperboard coatings and recycling methods on bacterial growth is demonstrated. The number of bacteria obtained on recycled fibers is affected by the presence of nanoparticles in coatings (Zn, Si and Al), as well as by the presence of different components in the base paper.

**Keywords:** sustainability, paper, offset prints, microorganism, bacteria

## 1. Introduction

Paper as one of the materials with the highest recycling rate is a sustainable material source for the cyclical manufacture of paper- and board-based packaging. The most important process in the production of recycled paper is deinking. Deinking is dependent upon the quality of the used paper for recycling, the type and properties of the printing inks and printing process, the age of the printed product and climatic conditions during its life cycle (Vukoje and Rožić, 2018). In order to achieve desirable optical and mechanical properties of recycled pulp, improved deinkability of printed paper products is an essential factor, and therefore different deinking processes can be used for the production of recycled fibers (Vukoje and Rožić, 2018). Due to the growing demand and increasing use of printed packaging materials such as cardboard, packaging grades classification regarding deinkability is also of great importance (Blanco, Miranda and Monte, 2013). Moreover, due to increased paper recycling rates, the quality of the waste paper used for recycling decreases since different paper fractions for recycling are being collected resulting in increased content of harmful substances (Pivnenko, Eriksson and Astrup, 2015). Thus, the evaluation of the quality of recycled paper should be considered. Pivnenko, Eriksson and Astrup (2015) showed a list of 10 000 chemicals potentially present in paper products, of which 157 were classified as hazardous. Some of these substances are associated with the printing industry or they originate from contamination of the paper during the use phase or during collection and handling in the waste management phase. Some of the present chemicals very likely remain in the solid matrix during paper recycling, and thus they may end up in new recycled products.

Paperboard packaging materials are finding increasing potential in packaging applications compared to classic polymer packaging materials, not only because of their environmental friendliness but also because of the fact that correctly stored paperboard materials when used in packaging reduce the potential of cross-contamination of food due to a quicker viability loss by spoilage

and pathogenic microorganisms, except for molds, compared to the polymer packaging according to Siroli, et al. (2017).

Due to increasing demand for environmentally friendly packaging materials, producers are obliged to use recycled fibers in their packaging manufacture, as well as other biodegradable materials. Different authors have been pointing to the problem of using recycled materials for food packaging applications, especially in the case of paper packaging, due to their chemical, microbiological and toxicological impurity and potential for migration of contaminants into the foodstuff and possible risk to consumers (Sipiläinen-Malm, et al., 1997; Escabasse and Ottenio, 2002; Pivnenko, Eriksson and Astrup, 2015).

Sipiläinen-Malm, et al. (1997) isolated aerobic spore-forming bacteria from recycled fiber pulp, some of which were considered pathogenic. Johansson, et al. (2001) showed that recycled papers are the source of microbial contamination that in the end may affect the purity of the paper. Moreover, the total amount of bacterial and fungal spores present in the paper is correlated with the amount of used recovered material as well as the season of production. Hladíková, et al. (2015) showed that by the increase of the recycled fibers content, the number of bacteria in the paper samples increased, while the molds were isolated only in a few paper samples. Additionally, it was concluded that microbial contamination of paper-based packaging containing recycled fibers may potentially cause a health safety risk (Hladíková, et al., 2015). Likewise, Namjoshi, et al. (2010) showed a positive correlation between recycle content and bacterial populations. Additionally, they found that bacteria persist in paperboard over long periods and may reenter the recycling process. According to Johansson, et al. (2001), the possibility of microorganisms and their metabolites migrating from the paper into food is dependent on humidity, pH, temperature and amount of fat or salt. Such migration can be overcome by the use of functional barriers between recycled packaging paper and food (Johansson, et al., 2001). Flemming, Meier and Schild (2013) and Zumsteg, Urwyler and Glaubitz (2017) showed the presence of microbial contamination in paper production, which may lead to economic losses, deterioration of raw materials and lowering product quality.

Even though the re-use of paper increases the microbial charge in the industrial process, the industrial process decreases the number of food-borne pathogens in the paper effectively. Thus, *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella* or confirmed coliform bacteria (Namjoshi, et al., 2010) and *Enterococcus* (Johansson, et al., 2001) were not found in any recycled paperboard product. Despite that, a possible negative impact caused by the remaining microbes on the human health could not be fully excluded (Johansson, et al., 2001). Additionally, Johansson, et al. (2001) reported the presence of endotoxins in recycled material, which was correlated with the amount of recycled material as well, indicating that endotoxins released during paper production and recycling might pose an environmental health problem.

Most microorganisms are harmless, various of them are used in biotechnology for the production of metabolites, enzymes and diseases protection inoculum. Microorganisms are used for bioremediation or fermentation in food processes. Some microorganisms or their enzymes have been used also in pulp and paper industry, such as cellulases and xylanases, which are economically and environmentally friendly. Pathogenic microorganisms like bacteria, viruses, fungi, protozoa, and multicellular parasites can cause diseases for humans and animals, as well economic and environmental consequences.

Considering the fact that the use of recovered paper increases the presence of microorganisms in the industrial process, which in the end has a negative impact on product purity and quality (Johansson, et al., 2001), the focus of this research was on the effects in industry related to a way that ensures product and consumer safety, to present the microbiological purity of recycled fibers derived from different samples, i.e. showing how different printing substrates, inks, nanomodified coatings, and recycling methods, influence the microbial growth on recycled fibers.

## 2. Experimental

### 2.1 Materials and preparation of the samples

Microbial purity of recycled paper samples obtained from recycling of different print samples and by different recycling methods was estimated in this study (Table 1).

For the preparation of cyan offset prints, white woodfree uncoated paper made from virgin fibers was used. Cyan offset prints were prepared using the Prüfbau Multipurpose Printability Tester simulating dry offset process. Test strips were printed in the full tone. Although this method introduces ink, and the impact on microbial growth is studied, it lacks the practical presence of fountain solution, which in reality can be a major source of contamination. Printed test strips were mixed with the unprinted paper samples, i.e. when the samples were recycled the total area of print in the recycling mixture was 37 % when com-

pared to the total area of used paper. Prepared cyan offset prints were recycled by means of INGEDE 11 method (INGEDE e. V., 2012) in combination with ultrasound (Badelin Sonoplus HD 3100 with a frequency of 20 kHz) pre-treatment for a duration of 10 minutes and amplitude 70 %. Additionally, enzymatic treatment was applied (BLX 14168, 1-4-endoglucanase, Buckman) instead of chemicals but adopting the same experimental procedure described in the INGEDE 11 method. The enzymatic recycling was performed at 45 °C, pH 7 with 0.125 % enzyme dosage calculated on the basis of oven-dry samples weight (Verma, Bhardwaj and Singh, 2017).

For the examination of microbiological contamination of recycled fibers made from the recycling of paperboard coated with polycaprolactone (PCL) coating and PCL nanomodified coating, the paperboard made from recycled fibers (230 g/m$^2$, GD2 grade, Umka color) was used. The middle layer of the used paperboard consists of mixed wastepaper and the top layer from sorted white wastepaper. The board is triple coated on the top side and single coated on the back side. The paperboard samples were printed by the offset printing process and coated with PCL coating and PCL nanomodified coating prepared for the improvement of packaging applications, as presented in research by Bota, et al. (2017) (Table 1). The coating was prepared from PCL biopolymer (Aldrich), dissolved in ethyl acetate while heated at 40 °C and stirring about 30 min to obtain 10 % homogeneous solution, using a magnetic stirrer. The PCL nanocomposite coatings were further prepared by dispersing nanoparticles with disperser (IKA T25 digital TURRAX) for 8 min at 15 000 rpm. The nanoparticle amount was calculated on the basis of PCL mass fraction: ZnO and $Al_2O_3$ were added in a portion of 1 % (samples GC2/PCL/1 % Zn and GC2/PCL/1 % Al) while $SiO_2$ was added in a portion of 2 % (sample GC2/PCL/2 % Si). The coating was applied using K202 Control Coater in controlled conditions defined by the ISO 187:1990 standard (International Organization for Standardization, 1990). Paperboard samples used in the recycling process were 100 % printed and coated, when compared to the total area of used paper. Prepared paperboard samples were recycled by means of INGEDE 11 method.

During recycling process of printed samples, all the chemicals and their dosages were in accordance to defined procedure described in INGEDE 11 method, except in case of enzymatic treatment. From the prepared pulp suspension, recycled laboratory paper handsheets were prepared on sheet former Rapid-Köthen Sheet Machine (PTI), according to standard method ISO 5269-2: 2004 (International Organization for Standardization, 2004). For each recycling process tap water was used, since the flotation process requires at least 12 L of pulp suspension, while during the handsheet preparation at least 8 L of water is required for just one laboratory paper handsheet production. Thus, the sheet former is connected to tap water supply system. During production of laboratory paper handsheets, the drying process was used, in which the wet pulp, placed between carrier board and cover sheet, was subjected to heating and vacuum drying at 92 °C for 5–7 min.

After each recycling, all the equipment used in the process was adequately cleaned and washed using washing chemicals and large amounts of hot tap water, previously chlorinated. Standard sterilization of the equipment requires sterilization at high temperatures and pressures, which in this case, was not possible due to the size of the devices, while the sterilization with strong chemicals could adversely affect the effective-

*Table 1: Samples and recycling methods used for the production of recycled paper*

| Samples abbreviation | Samples used for production of recycled paper samples | Recycling method |
|---|---|---|
| OFFSET-ENZYME | Cyan offset prints (120 g/m$^2$) | Modified INGEDE 11 + enzyme |
| OFFSET-INGEDE 11 | Cyan offset prints (120 g/m$^2$) | INGEDE 11 |
| OFFSET-INGEDE 11 + ULS | Cyan offset prints (120 g/m$^2$) | Modified INGEDE 11 + ultrasound |
| GC2 | GC2 uncoated paperboard (230 g/m$^2$) | INGEDE 11 |
| GC2/PCL | GC2 PCL coated paperboard (230 g/m$^2$) | INGEDE 11 |
| GC2/PCL/1 % Zn | GC2 paperboard with PCL coating modified with 1 % Zn nanoparticles (230 g/m$^2$) | INGEDE 11 |
| GC2/PCL/2 % Si | GC2 paperboard with PCL coating modified with 2 % $SiO_2$ (230 g/m$^2$) | INGEDE 11 |
| GC2/PCL/1 % Al | GC2 paperboard with PCL coating with 1 % $Al_2O_3$ (230 g/m$^2$) | INGEDE 11 |

ness of the devices. Thus, the step of devices sterilization is the limiting factor of this research. Additionally, the limitation of the study is microbiological check of recycling devices since this step was not conducted as well.

### 2.2 Methods for microbiological testing

When planning a microbiological test experiment, firstly the suitable test methods should be chosen. In this study, two methods were used, the smear method and the disintegration method. It is important to emphasize that sterile technique for determination of microbiological growth was used (sterile test tubes, sterile rods, sterile pipettes, sterile Ringer solutions, sterile knife for cutting the paper).

The microbiological testing of the recycled paper laboratory samples was not done immediately after the recycling process was conducted. The samples were tested after some time. But before research was conducted, all samples were preconditioned in the same laboratory conditions for 48 h (23 ± 2 °C, 45 ± 3 % RH) on air. The samples used were not tested for microbiological purity prior to recycling.

#### 2.2.1 Examination by the smear method

Sterile test tubes with sterile Ringer's solution were used for the preparation of different dilutions ($10^{-1}$ to $10^{-10}$). A horizontal method of sampling from the surface by using contact was used for the estimation of total number of bacteria and molds in recycled papers, by means of a sterile swab wetted in Ringer solution. As a swab material a commercially available sterile swab placed in sterile polypropylene tube was used. For each test, new sterile swab was used. It means, the swab is free from bacteria or other living microorganisms before sampling the studied samples, and free from any chemicals, bleaching agents, etc. The swab material was not treated with any chemicals or any other sterile technique before or during sampling. After sampling of samples surface, a swab was taken and placed in the sterile test tube, mixed for 30 s and separated by 1 mL of sterile pipette and transferred to another sterile $10^{-1}$ dilution tube. This procedure is repeated until $10^{-10}$ dilutions are made. Then, 1 mL of each dilution was placed on nutrient agar Petri dishes with Plate Count Agar (PCA) for bacteria examination and Sabourad Dextrose Agar (SDA) for examination of molds (both commercially available from Komed, Sveta Nedelja, Croatia). The growth media used are non-selective and commonly used to assess or to monitor the "total" number of microorganisms. Petri dishes were incubated at 37 ± 1 °C for 48 ± 3 h for bacteria and 30 ± 1 °C for 5 days for molds under aerobic conditions. After incubation, the total number of colony-forming units (CFU) was estimated.

#### 2.2.2 Examination by the disintegration method

The second microbial purity test was performed using the disintegration method, where 0.1 g of the test paper was disintegrated in a sterile Ringer's solution to obtain a fiber suspension. Recycled laboratory paper samples were disintegrated in the sterile test tube, with presterilized rod. Test tubes with sterile Ringer's solution were prepared and used for the preparation of different fiber suspension dilutions, which were afterwards plated on nutrient agar on Petri dishes with PCA for bacteria examination and SDA for examination of molds. In particular, 1 mL of each dilution was placed onto the Petri dishes and incubated at 37 ± 1 °C for 48 ± 3 h for bacteria and 30 ± 1 °C for 5 days for molds under aerobic conditions. After incubation, the total number of CFU was estimated and the concentrations of bacteria and molds per 1 g of paper were calculated. The experiments were carried out in triplicate. The average values are presented in this study.

## 3. Results and discussion

Only the total number of bacteria estimated by the disintegration method is shown since the smear method did not produce satisfactory results, i.e. no growth of microorganisms occurred. The similar result was obtained by Guzińska, Owczarek and Dymel (2012) who studied different methods of identifying microbiological contamination of paper and paperboard evaluation (defibering and smear). Despite the fact that smear method is standard procedure in microbiology, from this research and study by Guzińska, Owczarek and Dymel (2012) it can be assumed that this method has some limitations when used in sampling of recycled paper. The problems related to that may have been caused by the presence of the bacteria in the deeper layers or the absence of smooth surface, making the smearing sampling difficult. However, the smear method showed good results when materials containing a water impermeable aluminum foil or plastic coats were examined for microbiological contamination (Guzińska, Owczarek and Dymel, 2012).

During production of laboratory handsheets, the drying process was used, in which the whole laboratory handsheet was subjected to heat not only the surface. Additionally, the surface was not in direct contact with the machine since the pulp was placed between carrier board and cover sheet. Since the whole handsheets were subjected to heat, it could kill the microbes not only on the surface, but inside as well. In addition, the heating process is not expected to kill all the microbes since the complete sterilization process occurs at the temperatures above 120 °C for a minimum duration of 20 min. Despite drying process, moisture is always

present until the sample is fully dry, and so moisture can be present on warming conditions, ideal for microbial growth internally in the paper, which is the last part to become heated. Moreover, the microbiological tests were not performed right after the production of laboratory handsheets. Before the handsheets were tested for microbiological properties, they were placed in the laboratory for 48 h, at the same conditions, on air. During that period the laboratory handsheets were exposed to other external influences (microbes from the air) since they were not stored in sterile conditions.

Within this research, disintegration method showed good results and it was used for identification of microbiological contamination of recycled paper samples. Additionally, no molds or yeasts growth was observed within this experiment, only bacteria. In this study, the microbial growth on different recycled paper samples was studied, as well as the influence of different printing substrates (offset paper and paperboard), different recycling methods (INGEDE 11, INGEDE 11 in combination with ultrasound pretreatment and enzymatic recycling) and the presence of PCL nanomodified paperboard coating on bacterial growth.

Figure 1 shows the influence of cyan offset prints recycling method on microbial purity of recycled fibers. By comparing the obtained results, it can be seen that recycling method affects the number of microorganisms present in the samples. The largest number of microorganisms was isolated in the sample obtained by recycling with the INGEDE 11 method and ultrasound, which may indicate that the recycled fibers contain fewer toxic components that can inhibit the growth of microorganisms. Since in this part of the study, the same sample (cyan offset prints) were used for the recycling, it can be concluded that chemicals and pretreatment methods may affect the microbial purity of recycled fibers. It can be noticed that enzymes in paper recycling process contribute to the reduction of the number of microorganisms and the mentioned method of recycling is the most favorable in the terms of microbial purity (Figure 1). In addition, during the enzymatic treatment, recycling takes place under mild conditions (neutral pH, lower temperature) but during chemical deinking and ultrasonic pretreatment, some extreme conditions occur due to appearance of cavitation. Ultrasonic pretreatment thus more likely causes the stronger damage of cellulose fibers probably resulting in cellulose crystallinity decrease. In addition, the use of alkaline condition in ultrasonic treatment and conventional INGEDE 11 method, causes the higher rate of cellulose crystallinity decrease (increase of amorphous cellulose) compared to enzymatic treatment (Pathak, Bhardwaj and Singh, 2011; Sumari, Roesyadi and Sumarno, 2013; Kumar and Dutt, 2021). Moreover, it is known that amorphous parts of the cellulose are more susceptible to bacteria growth than crystalline regions, thus the lower number of bacteria for samples made using enzymatic recycling process can be found in comparison to samples made from conventional chemical and ultrasonic recycling (van Wyk and Mohulatsi, 2003). Moreover, it is important to emphasize that for the evaluation of microbial abundance in the tested samples in this study, dilution experiments were used. Each sample was tested three times, and for every tested sample, the series of dilutions were made. For this reason, the results uncertainty and somewhat higher statistical errors probably occur from the used methodology and nonrandom spatial distribution of the bacteria in dilutions.

The growth of bacteria on cellulose fibers after recycling of offset prints can be seen in Figure 2.

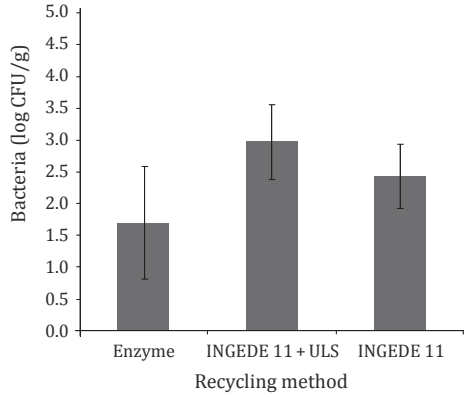


*Figure 1: Influence of the offset prints recycling method on the total number of bacteria*

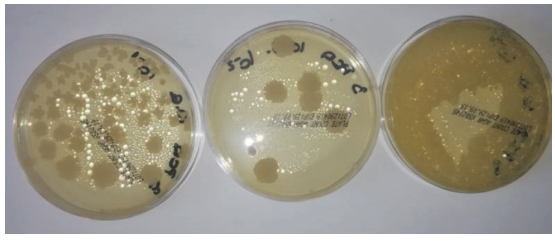

*Figure 2: Example of bacterial growth on coated paperboard in different dilutions*

Even though the best microbiological properties of the recycling procedures are obtained by the enzymatic deinking (Figure 1), for the evaluation of microbial purity of recycled paper obtained from recycling of paperboards, INGEDE 11 method was used for their recycling due to its frequent use in the paper industry. The use of INGEDE 11 method indicates how printed products will perform in an industrial deinking operation and is widely used by the paper industry and by many stakeholders in the paper value chain. This is the reason why it was used as a model

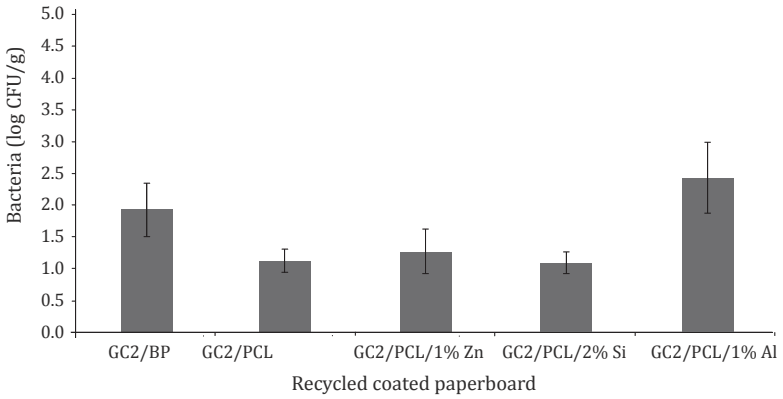


*Figure 3: Influence of the coating formulation on paperboard surface on the total number of bacteria*

for development of standardized procedure to simulate the principle process steps for ink detachment and ink removal under standardized alkaline conditions at a laboratory scale, namely in ISO 21993:2020 (International Organization for Standardization, 2020).

The results show that the presence of PCL coating and PCL coating modified with nanoparticles in wastepaper can affect the microbial purity of recycled fibers (Figure 3). Smaller number of bacteria was observed for the samples containing PCL coating and PCL coating modified with Zn and Si nanoparticles, which means that they show antibacterial properties. Al nanoparticles do not show significant antibacterial properties, unlike Si and Zn nanoparticles, probably due to the different properties of nanoparticles and their interaction with bacteria.

According to Zhang, et al. (2014) aluminum has no antibacterial function. Additionally, there is a possibility that nanoparticles were washed out into the wastewater stream during paper recycling resulting in lower degree of antibacterial properties.

The study of PLA coated paper containing ZnO nanoparticles recycling (Zhang, et al., 2016) showed that 86–91 % ZnO nanoparticles ends up in the rejected material stream, mostly embedded within the polymer coating, while 7–16 % nanoparticles end up in the accepted fiber material stream. The lack here is that their presence in the process water cannot be completely ruled out since their concentration was not directly measured. The authors are of the opinion that nanoparticles may accumulate in the white water system during paper recycling process due to coating fragmentation and migration to water streams.

The antibacterial properties of nanoparticles are due to their small size, i.e. nanoparticles can behave as molecules when interacting with a cell, which allows them to easily penetrate the cell membrane and interfere in vital molecular pathways if the chemistry is possible (Nastulyavichus, et al., 2019). ZnO nanoparticles exhibit strong antibacterial property over a broad range of microorganisms, i.e. ZnO shows more pronounced effect on Gram-positive (*Staphylococcus aureus*) than Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria, and the bactericidal efficacy was found to increase by decreasing the particle size (Yemmireddy and Hung, 2017). According to Jia, et al. (2019) antibacterial and antifungal activity of ZnO nanoparticles is related to the release of $Zn^{2+}$ ions, and mainly caused by the attachment of ZnO nanoparticles to the bacterial cell wall.

The antibacterial effect of the Si-nanoparticles can be assigned to their attachment on the bacterial outer wall resulting in the mechanical damage of the bacterial membrane related to the oxidative effects of the amorphous Si nanoparticles generated singlet oxygen and reactive oxygen species or due to photodynamic inactivation by the singlet oxygen, that is usually generated on the surface of Si nanoparticles during their production in water and alcohol, which leads to significant oxidative damage of the biological object and DNA damage (Smirnov, et al., 2018). Due to silanol groups interaction with bacterial wall's functional groups by hydrogen bonds, destabilization of the peptidoglycan (bacterial wall) occurs (Mustafa, 2018).

Components present in paper (fillers, chelating agents, etc.) and printing inks and their constituents (i.e. heavy metals) may affect the development of microorganisms, i.e. they may show antimicrobial properties (Muñoz-Bonilla and Fernández-García, 2012; Lemire, Harrison and Turner, 2013). The results show that a higher number of bacteria developed on recycled paper samples made from offset prints compared to recycled samples made of nanomodified coated paperboard (Figure 4). This behavior is likely to arise from two possible scenarios. First, the paper used for cyan offset prints, itself is cleaner than the paperboard

used for printing and coating with PCL nanomodified coating. Therefore, there is a smaller number of toxic components as well. Second, the recycling of cyan offset prints was made in such a way that the total print coverage was 37 % of the total weight of the recycling paper (the rest was unprinted paper). When recycling nano modified coated paperboard, 100 % of the prints were recycled (paper was not mixed with unprinted paper). This would mean that a small amount of the printing ink present in the prints has a negative effect on the development of the bacteria, i.e. the constituent components of the printing ink are likely to be toxic to bacteria.

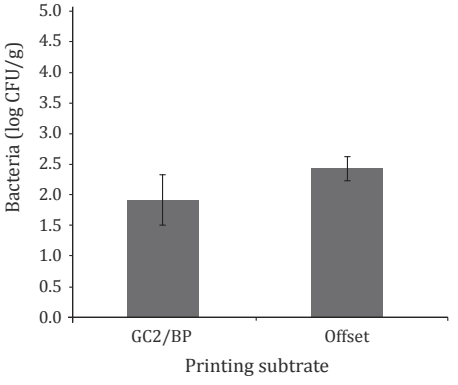


*Figure 4: Influence of printing substrate on the bacterial growth (GC2/BP – uncoated paperboard, offset – offset paper) recycled by means of INGEDE 11 method*

When talking about microbial purity of recycled fibers, it is generally necessary that the total counts of yeasts, molds and bacteria must be as low as possible without presence of pathogenic bacteria, including *E. coli* and other enterobacteria (Suihko and Skyttä, 1997). It should be noted as well that some of the pathogenic microorganisms in the recycled and virgin paper pulp can be destroyed in the process of paper bleaching by the action of oxidizing agents or due to high temperatures present for example in the repulping process and paper drying.

## 4. Conclusions

The results of this study show that disintegration method is suitable for the isolation of bacteria from recycled paper, unlike smear method. The possible explanation of the obtained results probably lies in the fact that inner parts of the paper (deeper layer of paper) due to higher moisture content contain higher number of bacteria. Moreover, it is possible that the smear method has limitation when used in paper sampling due to rougher surface. Additionally, the disintegration method used was only valid for isolation of bacteria because no molds or yeasts were found. Obtained results indicate that paper components can influence the bacterial growth. Paperboard, which is made of recycled fibers and therefore contains a certain number of toxic components acting against bacterial growth, will give a smaller number of bacteria than offset paper. Additionally, a greater amount of printing ink in the samples may have an antibacterial effect as well. The recycling method itself will also affect the growth of bacteria, i.e. cleaner fibers will provide more bacteria. With nanomodified PCL coatings, the nanoparticles themselves can also act antibacterial. Pathogenic microorganisms in the recycled and virgin paper pulp can be destroyed in the process of paper bleaching by the action of oxidizing agents or due to high temperatures present in repulping process and paper drying. Moreover, the process of paper drying can have some influence on the microbiological growth. In the future, research should be conducted on different nutrient media to determine whether their composition has an impact on bacterial growth. Additionally, it is important to emphasize that the paper samples used were not tested for microbiological purity prior to recycling, and thus it can be assumed that microbial presence after a period of time following recycling could have originated from a range of contamination sources. Thus, all the future research should involve the study of incoming paper samples prior to recycling in order to get a better insight into the bacterial contamination of recycled paper samples and the influence of paper components on microbial growth.

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