BIODEGRADATION STUDY OF RVNRL, LEFFECT OF BACTERIA ON THE QUALITY OF RVRNI FILM.

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BIODEGRADATION STUDY OF RVNRL, 1. EFFECT OF BACTERIA ON THE QUALITY OF RVRNL FILM

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ABSTRACT

BIODEGRADABILITY STUDY OF RVNRL, 1. EFFECT OF BACTERIA ON QUALITY OF RVNRL FILM. Biodegradation of Radiation Vulcanisation Natural Rubber Latex (RVNRL) film by using bacteria's of *Alcaligenes faecalis*, *Mycobacterium fortuitum* and *Staphylococcus epidermidis,* has been studied. The objective of the investigation is to study the degradability of RVNRL film by using bacterias with biodegradable ability. Sample of RVNRL film inserted in the reaction tube, which consists of medium and bacteria, were incubated in a desicator at room temperature and fixed time. After that the samples were washed, dried, weighed and measured for their mechanical properties. The results show that the samples, which were incubated for 7 weeks in combination with *A. faecalis, M. fortuitum* and *S. epidermidis*, can sharply decrease the mechanical properties (elongation at breaks, hardness and tensile strength). Meaning that RVNRL film could be considered as biodegradable material.

Key words; RVNRL, *A. faecalis, M. fortuitum* and *S. epidermidis*

ABSTRAK

STUDI BIODEGRADASI LAVR, 1. EFEK BAKTERI PADA KUALITAS FILM LAVR. Telah dipelajari biodegradasi film Lateks Alam Vulkanisasi Radiasi (LAVR) dengan bakteri *Alcaligenes faecalis, Mycobacterium fortuitum* dan *Staphylococcus epidermidis*. Tujuan penelitian yaitu ingin mengetahui degradabilitas film LAVR dengan memanfaatkan kemampuan bakteri tersebut. Contoh film LAVR dalam tabung reaksi yang berisi media dan bakteri, diinkubasi dalam desikator pada suhu ruang dan waktu tertentu. Setelah itu contoh dicuci, dikeringkan, lalu ditimbang beratnya dan diukur sifat-sifat mekaniknya. Hasilnya menunjukkan bahwa kondisi inkubasi selama 7 minggu serta kombinasi bakteri *A. faecalis, M. fortuitum* dan *S. epidermidis*, dapat menurunkan secara tajam sifat-sifat mekanik (perpanjangan putus, kekerasan dan tegangan putus). Hal ini menunjukkan bahwa contoh film LAVR adalah bahan yang *biodegradable*.

Kata kunci; LAVR, *A. faecalis, M. fortuitum* dan *S. epidermidis*.

INTRODUCTION

Radiation vulcanisation of natural rubber latex (RVNRL) means cross linking of natural rubbers (cis-1,4-polyisoprene) dispersed in aqueous medium. Unvulcanized rubber is generally not very strong, could not maintain its shape after large deformation. Useful rubber articles, such as gloves and condoms cannot be made without vulcanization. RVNRL can be achieved without the presence of sensitizer (1). Sensitizer are defined as chemicals able to reduce the vulcanisation dose (Dv ; dose at which the maximum tensile strength of dried film of irradiated latex is found). Initially, RVNRL was accomplished by irradiating natural rubber latex without sensitizer using more than 300 kGy, which is too high to be used for industrial application. At present, n-BA is considered to be the most effective sensitizer regarding to the efficiency of radiation vulcanisation e.g. low Dv and high maximum tensile strength. But n-BA possesses some disadvantages such as bad smell and destability of latex. Nevertheless RVNRL has many advantages over the conventional sulfur vulcanised latex, such as absence of nitrosamine, very low cytotoxicity, low emission of gases when incinerated and good degradability (2, 3).

Fresh natural rubber latex (*Hevea brasiliensis*) contains about 30 % rubber hydrocarbon, which is cis polyisoprene and about 4 to 5 % non rubber solids comprising mainly of proteins, lipids, carbohydrates and mineral salts. Each latex particle is bound by a membrane of phospholipid and protein, which protects the latex particle (4). There are several protein present in fresh field latex and the major one are alpha globulin, albumin and hevein. The protein content of field latex is approximately 1 % of the weight of fresh latex. About 20 % of the protein is absorp by the rubber particles and the remainder is in the serum phase. The total lipid content of natural rubber latex is around 1 % but can vary from as low as 0.5 % to as high as 1.6 % 0f the latex. The lipids are neutral lipids (50 %), glycolipids (30 %) and phospholipid (20 %). The main carbohydrate present in field latex is quabrachitol which is present up to 2 % and sucrose is present at 0,1 % to 1 %. These carbohydrates being in the serum are washed away during processing. However the sugar in the glycolipids, mainly galactose and some glucose and inositol will still be present in the rubber. Minerals such as potassium and magnesium and phosphate are present at approximately 0,5 % of latex (5). These conditions are good medium for supporting the growth of microbes, so to enhance the degradability of RVNRL film using the bacteria is one of the method needed.

The investigation carried out is to study whether the bacterias *A. faecalis, M. fortuitum* and *S. epidermidis* have an effect on the degradability of RVNRL. It is known that

bacteria *A. faecalis* are usually active as an organic material decomposer. *S. epidermidis* is a bacteria which is able to produce lipase (glyserol ester hydrolase) an enzyme able to synthesis lipid to di or monoglyserida and lipid acids. *M. fortuitum* is able to produce proteinase enzyme, which could hydrolyse protein molecule to more simple compounds (6).

The hypothesis of this experiment is to prove that the RVNRL film is biodegradability material and the combination with bacteria of *A. faecalis, M. fortuitum* and *S. epidermidis* has the capability to degrade the rubber film.

MATERIALS AND METHOD

Materials. The natural rubber latex, used in this experiment is obtained from Sukabumi, West Java with total solid content (TSC) about 61 % and 60 %. dry rubber content. Latex is dissolved by ammonia solution about 1,6 % up to 50 % from the total solid content. The monomer of n-butyl akrylate (n-BA) was used as sensitizer. Medium of Tryptone Soya Agar was used to cultivated the bacteria. The chemical materials of K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2$, $FeSO_4.7H_2O$ and $ZnSO_4$ were used as medium to incubate the film and bacteria. The chemical material is technical and used directly without purification, except elimination of inhibitor, which was pro, analyse. Samples of the bacteria's i.e. *A. faecalis, M. fortuitum* and *S. epidermidis* were obtained from the Center for Research and Development, Office of Veterinary Research, Bogor.

Equipment. The latex irradiator of ^{60}Co with a capasity of 190.000 Curie is used as a irradiation source. Instron Testing Machine is used to measure the mechanical properties of RVNRL film and desicator are used to incubate the RVNRL film.

Latex concentration. 100 kg latex which was just tapped is given 1 % primer preservation of liquid ammonia, then brought to factory for latex concentration. Latex was centrifuged to be concentrated at a speed of 7.000 rpm for 2 hrs. The centrifuged latex is given a secondary preservation material directly, while stirring it slowly. Preservation material, used were ammonium laurat 0,2 phr (per hundred rubber) and ammonia 0,7 % weigh of latex. Furthermore latex, which has been given the secondary preservation, was poured in plastic bottles and can be storaged for 1-2 months (7).

Process of Vulcanization Radiation Natural Latex. About 2 liter of natural concentrated latex, which was given preservation material, and then added with nBA sensitizer, as much as 2 phr in emulsion form. After that latex was stirred slowly and irradiated at a dose of 35 kGy and with a dose rate of 2 kGy/hrs. During irradiation latex was placed in containers without stirring.

Sample preparation. The irradiated RVNRL was poured into the glass plate to obtain latex film, which will be measured for its mechanical properties. After dried at room temperature, the film was washed in boiling water to eliminate the remaining ammonia, and then dried in the oven at 70 °C for 1 hour. Whereas the sample of bacteria's were cultured on medium of Tryptone Soya Agar, and incubated in an incubator at optimal temperature for 24 hours. After incubation the cells were harvested and washed with sterile aquadest and centrifuged at 3.000 rpm for 30 minutes. The cells, which have been cleaned, hereinafter were adjusted to concentration of about 10^8 cells/ml. These cells will then be used in the biodegradability process of RVNRL film. The sample of the medium, was weighed, dissolved and heated on an electric heater. The dissolved medium was put into the reaction tubes, each tube was filled with 50 ml medium and then sterilized in an autoclave at 121 °C for 15 minutes.

Measurement of the properties of latex and natural rubber film. Tensile strength, modulus and elongation at breaks are measured by using Instron Testing Machine made in Toyoseiki Co. Ltd., Japan, at speed of crosshead 100 mm/minute and at 25 °C. Standard of the testing was coincided with method of ASTM D 412-87. While the TSC, DSC, viscosity, pH, MST, ammonia content, coagulum content were measured using ASTM D 1076-88, and the extracted protein content and also nitrosamine content were measured according to FDP procedure (8, 9).

Degradability testing. The rubber film which has been weighed straight after drying, and put into glass tubes with a 25 mm diametered which consisted of 50 ml medium and 0.2 ml cell of bacteria. The composition of the medium is K_2HPO_4 1,5 g, $MgSO_4.7H_2O$ 0,05 g, $CaCl_2$ 0,05 g, $FeSO_4.7H_2O$ 0,015 g, $ZnSO_4$ 0,005 g in 1 litter of aquadest (10), and pH was made up to 7. Afterward the sample of film samples were incubated in a desicator at room temperature for fixed time. After incubation, the samples were washed with aquadest and methanol, dried in a vacuum oven at 40 °C for 24 hours. Henceforth the film RVNRL was weighed, measured for tensile strength, elongation at break, modulus 300 % and modulus 600 %.

RESULTS AND DISCUSSION

Quality of RVNRL. RVNRL is a pre-vulcanised natural rubber latex prepared by radiation process which provides concentrated latex particularly suitable for dipping applications. No vulcanisation materials (curing ingredients) are required and hence vulcanising recipe has not to be considered. The typical properties of RVNRL produced by

Center for Research Development Isotopes and Radiation Technology – National Nuclear Energy Agency (P3TIR-BATAN) is shown in Table 1. Data in this Table indicate that RVNRL is a stable latex, transparency, low modulus, absent of nitrosamine and with low water soluble protein content.

Stable pre-vulcanised latex. RVNRL has better latex stability, this was shown that even with nine months the surface among rubber particle before incubation was more sticky than after storaged (Figure 1A and 1B). It means that the non-rubber particle substract such as protein, carbohydrate is degraded by self oxidation, and consequently the MST increases.

Transparency. Product from RVNRL should have high transparency, due to the absence of curing ingredients, and this is desirable for products such as teats, soothers, and some catheters. Additionally, their colour should be very pale since they do not need to be heated at high temperatures for vulcanisation-agent. This quality is much in demand for teats and soothers.

Low modulus and high elongation at break. The low modulus of RVNRL products are of some advantages for articles such as gloves and toy balloons, where a high modulus is not desirable.However, the cross linked density will need to be sufficient to prevent distortion of the product, because if the modulus too low it will be difficult to remove from the former.

Absence of nitrosamine. The absence of the accelerators (dithiocarbamates and thiazole) and sulphur from a radiation vulcanised latex ensure that products made from RVNRL will not cause any of the demartitic/allergic reactions associated with this chemicals. A part from demartitic (irritation of skin) the latex accelerators tissue irritation is also experienced by patients who have to have latex urinary catheters implanted for long periods.

Lower extractable protein content. MAKUUCHI et all. (11) reported that nitrogen contents (measured by Kjedehl method) in rubber film of RVNRL had higher amounts than that from non irradiated natural rubber latex. This is due to some radiolysis products of non-rubber (protein, lipids, etc.) formed ammonium salts. This means that protein in natural rubber become water-soluble by irradiation, consequently the water soluble content of RVNRL is lower. According to the recommendation of STIGI (12), the maximum water soluble content of one peace surgical glove is no more than 1,200 µg/glove or 120 µg/g. Table 1 shows that extractable protein content of RVNRL film was 110 µg. This means that RVNRL is not only used for producing catheter, and teat but also for surgical gloves.

. *Resistance of RVNRL film against bacteria.* The content of RVNRL film is not only a cross linked among of the rubber particles, but also non rubber particles such as proteins, lipids, carbohydrate, mineral salts, antioxidants (13). The physical and mechanical properties

of RVNRL before and after incubated in the medium, which content of bacteria *A. faecalis, M. fortuitum* and *S. epidermidis* and it combinations are shown on Table 2 - 4 and Figure 2. Table 2 shows that there is tendency in the increase of the film weight, which was incubated either without or with, cultured bacteria, this is due to sweeling of the film during incubation. This condition make possible could trapped the substrate in the porous of the film (Figure 1C and 1D), so that the weigh of the film increase. Figure 2X and 2Y show that the modulus 300 % and 600 % of film after incubation either without or with cultured bacteria do not show significant difference. It means that incubation of the medium either without or with cultured bacteria didn't effect of the changes of modulus 300 % and 600 %. Table 3, 4 and Figure 2Z show that of elongation at breaks, hardness and tensile strength of the film either without or with cultured bacteria is decrease. In general the mechanical properties (elongation at breaks, hardness and tensile strength) of RVNRL film were decreased, because the film were degraded by combination of bacteria, after incubated for 7 weeks.

CONCLUTION

From the result it can be concluded that the RVNRL film which incubated in the medium and the combination of bacteria i.e. *A. faecalis, M. fortuitum* and *S. epidermidis* for 7 weeks, the elongation at breaks, hardness and tensile strength of the film decrease. It means that the RVNRL film is biodegradable material.

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Table 1. The typical properties of RVNRL produce by P3TIR-BATAN

Properties		Value	
		Minimum	Maximum
Latex; - pH	A	10.4	10.7
	B	10.5	10.7
- Total solid Content (TSC), %.	A	55.6	55.8
	B	55.3	55.5
- Dry Rubber Content (DRC), %.	A	54.7	54.8
	B	54.6	54.7
- TSC-DRC	A	0.9	1.0
	B	0.7	0.8
- Brookfield Viscosity , cP. (spindle 18/30 rpm at 25° C)	A	40	60
	B	43	62
- Mechanical Stability Time (MST), Sec.	A	1200	1800
	B	1800	1800
- Ammonia Content, %.	A	0.7	0.7
	B	0.7	0.7
- Coagulum Content, %.	A	0.002	0.002
	B	0.002	0.002
Film; - Modulus 600 %, MPa.	A	2.4	2.7
	B	2.3	2.4
- Elongation at Break, %.	A	990	1100
	B	950	1000
- Tensile Strength, MPa.	A	24	27
	B	23	24
- Nitrosoamin Content, µg/g.	A	2	2
	B	1	1
- Extractable protein Content, µg/g.	A	90	110
	B	80	90

A = latex before incubation, B = latex after incubation for 9 months.

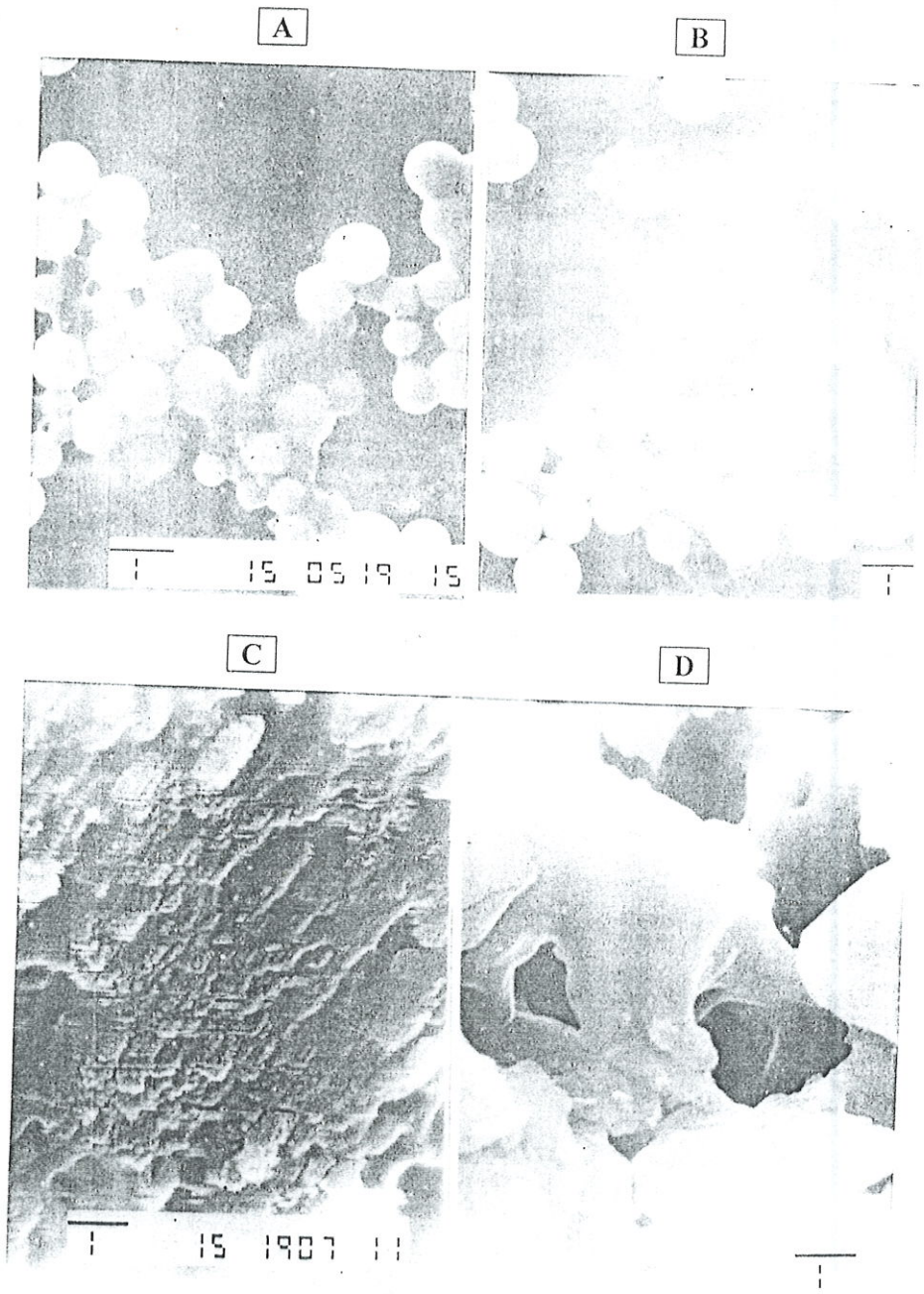

Figure 1. The Scanning Electron Microscope of latex particle (A - B) and RVNRL film (C - D) before and after storage in the room for 9 months

Table 2. Changes of weigh (% mg) rubber film after and before incubated in the medium which consisted of bacteria's *A. faecalis* (A), *M. fortuitum* (M) and *S. epidermidis* (S)

Incubation time (weeks)	Bacteria							
	Control	A	M	S	AM	AS	MS	AMS
0	0.25							
1	0.29	0.50	0.50	0.48	0.49	0.77	0.19	0.29
3	0.44	0.73	0.68	0.77	0.71	0.48	0.53	0.43
5	0.61	0.85	0.93	0.84	0.84	0.66	0.54	0.63
7	0.60	0.88	0.79	0.78	0.82	0.63	0.61	0.63

Table 3. Changes of elongation at break (%) rubber film after and before incubated in the medium which consisted of bacteria's *A. faecalis* (A), *M. fortuitum* (M) and *S. epidermidis* (S)

Incubation time (weeks)	Bacteria							
	Control	A	M	S	AM	AS	MS	AMS
0	1170							
1	950	880	1030	940	950	910	970	960
3	950	980	910	920	910	910	910	930
5	950	910	960	920	920	910	970	980
7	970	920	920	890	900	900	860	820

Table 4. Changes of hardness (Shore A) rubber film after and before incubated in the medium which consisted of bacteria's *A. faecalis* (A), *M. fortuitum* (M) and *S. epidermidis* (S)

Incubation time (weeks)	Bacteria							
	Control	A	M	S	AM	AS	MS	AMS
0	27							
1	25	25	25	25	23	23	23	22
3	25	25	25	23	23	23	23	22
5	25	24	25	24	23	23	22	22
7	24	23	22	22	23	23	21	21

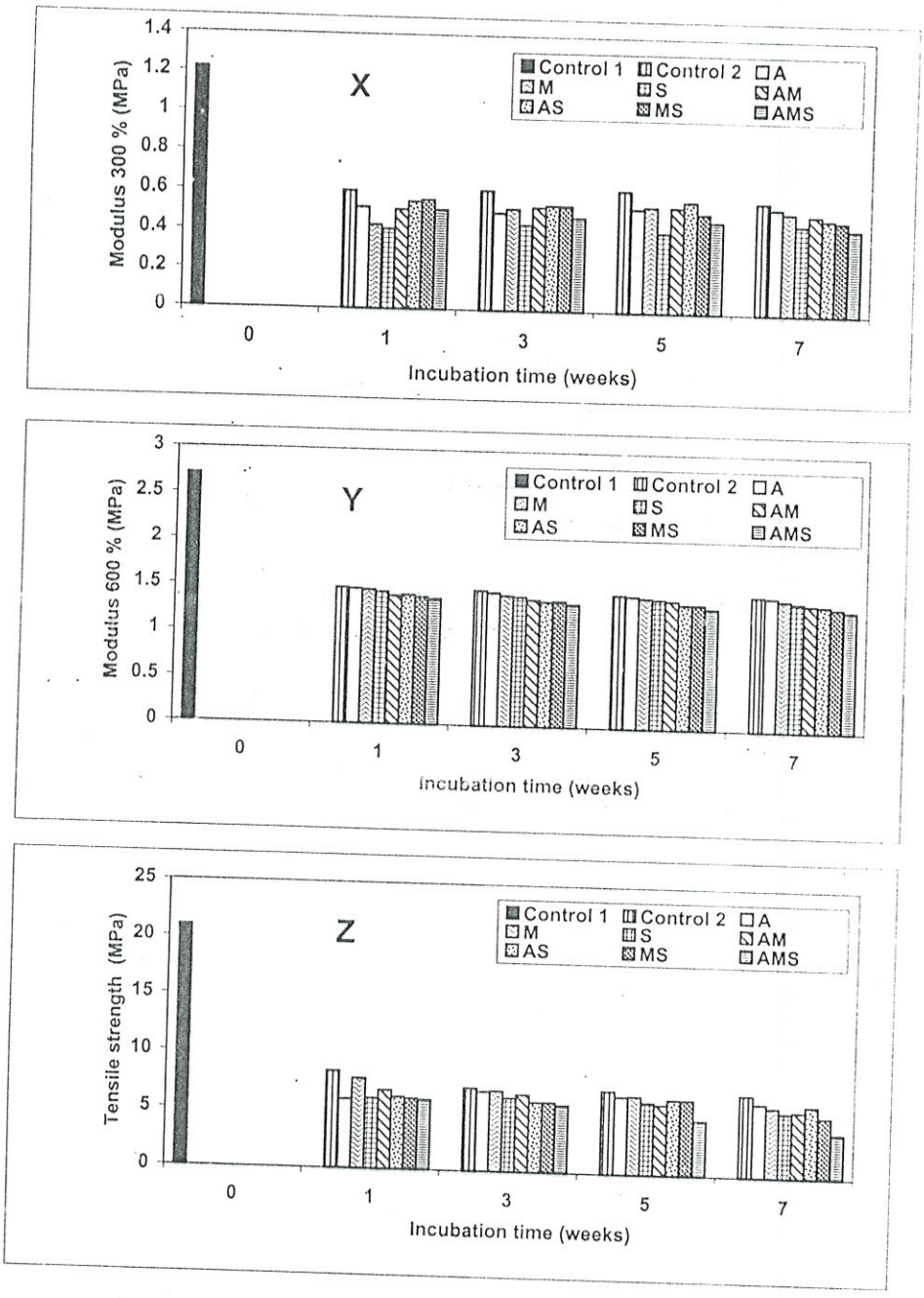

Figure 2. Changes of modulus 300 % (X), 600 % (Y) and tensile strength (Z) After incubated in the medium which cultured by bacteria's of *A. faecalis* (A), *M. fortuitum* (M) and *S. epidermidis* (S)

Information; Control 1 = sample before incubated in the medium and wrapping paper

Control 2 = sample after incubated in the medium, into paper bag, but without bacteria's