

## Development and Characterization of Ternary Hybrid Membranes in Natural Polymers and their Application in the Microbiology Field

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### Abstract

New ternary membranes have been synthesized and characterized. The membranes have been elaborated with natural polymers such as "corn starch" and "Bentonite" added with activated carbon. The technique of phase reversal induced by a solvent at ambient temperature has been applied for the development of these membranes. A set of characterization techniques which include FTIR, SEM are used for structural, morphological knowledge. The ternary membranes are performed on microbiological separation for the filtration of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*, *Penicillium notatum*) and compared to binary membranes which include natural polymers such as "corn starch" and "Bentonite" as compositions. Results showed that the ternary membrane has high performance compared to binary membranes for the filtration *Escherichia coli*, *Pseudomonas aeruginosa* and fungi *Aspergillus niger*.

**Keywords:** Natural polymers; Synthesis, Characterization; Bacteria; Fungi; Separation

### Introduction

Membrane technologies have been widely studied in recent years [1,2]. The key efficient and economical separation of this process is the membrane [3], which is defined as a physical barrier to retain or pass microorganisms. The most important desired characteristics of the membranes are the good permeability, high selectivity and stability [3-5]. Today, the membrane separation must now be considered as powerful advanced technologies and convenient to use [6]. Their installation and implementation requires attention and special expertise [7-9]. The application of membrane filtration technology in the field of microbiology requires the development of new membranes based on natural polymers such as corn starch and bentonite [10]. The use of a biopolymer such as starch can be an interesting solution because such a polymer is relatively inexpensive, abundant and biodegradable. The inversion technique of induced phase by solvent has been applied to the preparation of these membranes [11]. This technique is most suitable for our selected polymers [12,13]. Our work involves the development of two types of membrane, the binary starch and bentonite membrane and ternary membrane by adding commercial activated carbon to the first. The incorporation of the latter material was a need as there was increased retention of certain microorganisms selected during filtration.

### Materials and Methods

Natural powder of corn starch is extracted from maize raw material. Two types of commercial bentonite sodium were obtained from BENTAL Company "ENOF" of Mostaganem. A commercial activated carbon (REIDEL) was purchased from Maen (Germany) and the distilled water was used as solvent during membranes fabrications. Bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*) were obtained from laboratory hospital of Mostaganem, and fungi strains (*Aspergillus niger*, *Penicillium sp*) were obtained from microbiology laboratory, department of biology of Mostaganem/Algeria.

### Membrane preparation

The membrane preparation technique used in our work requires corn starch, bentonite and commercial activated carbon (REIDEL). A series of membranes with various proportions of these three materials was developed. The technique of induced phase inversion by solvent is

used to make an asymmetric support with thin layers and high porosity, this method allows the fabrication of flat asymmetric hybrid membranes at different concentrations of polymers and activated carbon. The selected suitable solvent was distilled water for reasons of cost, safety, availability and its chemical affinity with natural polymers. As the corn starch is insoluble in cold water, the corn starch prepared solutions are heated to a temperature of 70°C and stirred magnetically for a period of 90 minutes, after the solubility test of the corn starch, the selected solution in which the concentration is 25 g/L, this solution is called a starch paste. The incorporation of bentonite and activated carbon to the starch solution allows the preparation of ternary and binary mixture whose protocol is as follows. 20 ml of the prepared corn starch solution was added to different amounts of treated/untreated bentonite and various proportions of activated carbon. The mixture is heated under magnetic stirring for two hours to ensure solution homogeneity. 1 ml were collected from the mixture which then spread on a filter paper type Macherey-Nagel on circular diameter of 7 cm using a metal roll. The prepared membranes were dried in an oven and maintained at a moderate temperature, the membranes then wrapped in aluminum foil to sterilize those that have been applied to microbiological filtration. The characterization was performed on selected membranes after filtration microbiological tests.

### Microbiological separation

Strains of bacteria such as (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*, *Penicillium sp*) were activated and purified by Gram staining technique before any microbiological test filtration. All conditions were prepared in the laboratory using conventional microbiological techniques: the broth and nutrient agar for bacteria, broth and Sabouraud agar for fungus. After activation of

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the different strains, the bacterial suspensions and those of fungus were reactivated before each test with respecting of the filtration duration (24 to 72 hours) and the incubation temperature (27°C to 30°C). The filtration was carried out in the microbiology laboratory in well sterile conditions to avoid any air contaminations. After the filtration test on wide range of developed membranes, series membranes have been selected in which the bentonite concentration is 100 g/L and that of the activated carbon of ternary is 12.5 g/L. As our goal is to design selective polymeric membranes. The selected series of membranes present the retention property for the selected microorganisms.

## Results and Discussion

### Membrane characterization

The prepared membranes are characterized using two methods, the top surface morphologies of membranes were observed by scanning electron microscopy (Hitachi TM 1000) which can achieve 30000 nm of resolution. The chemical group an interaction is identified using Fourier Transform Infrared Spectroscopy (FTIR) (SHIMADZU IR Prestige-21), range of wave number is 400-4000  $\text{cm}^{-1}$ .

### Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy was utilized to identify vibration modes of different functional groups of prepared membranes  $M_1$ ,  $M_2$ ,  $M_3$  and  $M_4$ . The results are shown in Figure 1. As can be observed, the four membranes IR Specters are similar in the range of 4000-1000  $\text{cm}^{-1}$ , it seems that the peaks intensity decreases during the addition of activated carbon. The band intensity caused by the stretching vibrations Si-O-Si (1035 -1024-1037-1042) and the bands due to deformation vibrations Al-OH (915  $\text{cm}^{-1}$  for the membranes  $M_1$  et  $M_2$ ) were not affected by the addition of activated carbon as shown in Figures 1-4.

### Scanning Electron Microscopy (SEM)

As shown on Figures 5 and 6, the photos a, b, c and d corresponding to the membranes  $M_1$ ,  $M_2$ ,  $M_3$  and  $M_4$  shows that the surfaces have pores with size and distribution and shape more or less heterogeneous.

### Performance evaluation of ternary membranes compared to binary membranes

The microbiological experiments was used to test the membranes  $M_1$ ,  $M_2$ ,  $M_3$  and  $M_4$  the ability of these to filtrate the microorganisms;

*Escherichia coli*, *Pseudomonas aeruginosa* as bacterial test and *Penicillium notatum* and *Aspergillus niger* as a fungal strains. The microorganisms strains was delivered from the laboratory of microbiology; University of Mostaganem, Algeria, after isolation and preparation of the cultures at specified milieu the nutrient broth milieu for bacteria and sabouraud liquid for fungi. The results of the filtration of selected membranes  $M_1$ ,  $M_2$ ,  $M_3$  and  $M_4$  are grouped in three (Tables 1-4). The first experiment where the membranes  $M_1$ ,  $M_2$ ,  $M_3$ , and  $M_4$  have been tested on filtration of *Escherichia-coli* and *Pseudomonas aeruginosa* microorganisms, the binary membrane with treated bentonite  $M_1$  has retained the tow microorganisms, but the results were negatives with not treated membrane  $M_3$  due the effect of treated bentonite on structure of the membrane pore diameter. The retaining of *Escherichia coli* and *Pseudomonas aeruginosa* bacteria has been enhanced after adding the activated carbon on the membrane  $M_3$  for the results of the filtration. *Aspergillus niger* and *Penicillium notatum* in Table 4, the membranes  $M_3$  have showed a negative results contrary with bacteria showed in Table 3. For the membrane  $M_4$ , the retention of *Aspergillus* can be explained by the morphology of this fungi which has a significant mycelium that cannot be passing through diameter holes of the membranes  $M_3$  and  $M_4$ . The negative result of membranes  $M_1$ ,  $M_2$  may be due to the morphology spores released by the fungi.

## Conclusion

From this work, a ternary membrane  $M_2$ ,  $M_4$  were successfully prepared by inversion technique of induced phase by solvent, the fabricated membrane is shown a porous surface with heterogeneous morphology. The investigated ternary membranes  $M_2$ ,  $M_4$ , the membrane  $M_4$  which is the adding of the activated carbon on (Bentonite/corn starch) binary membrane results in enhanced on microbiological separation compared to binary membranes  $M_1$ ,  $M_2$ . The most important characteristics (pore diameter and porous volume) scanning electron microscopy observation showed homogeneous layers and porous without cracking; the same results were showed by Agoudjil et al. in 2014; where they obtained a silica membranes which can be used in Ultrafiltration [14]. We can say this work; is new approach to think about how we can use the "bentonite"; natural polymer "corn starch" and active carbone to prepare polymeric membrane to filtrate the microorganism; furthermore work is on to see permeability of the prepared membrane using other bacteria; fungi and, viruses.

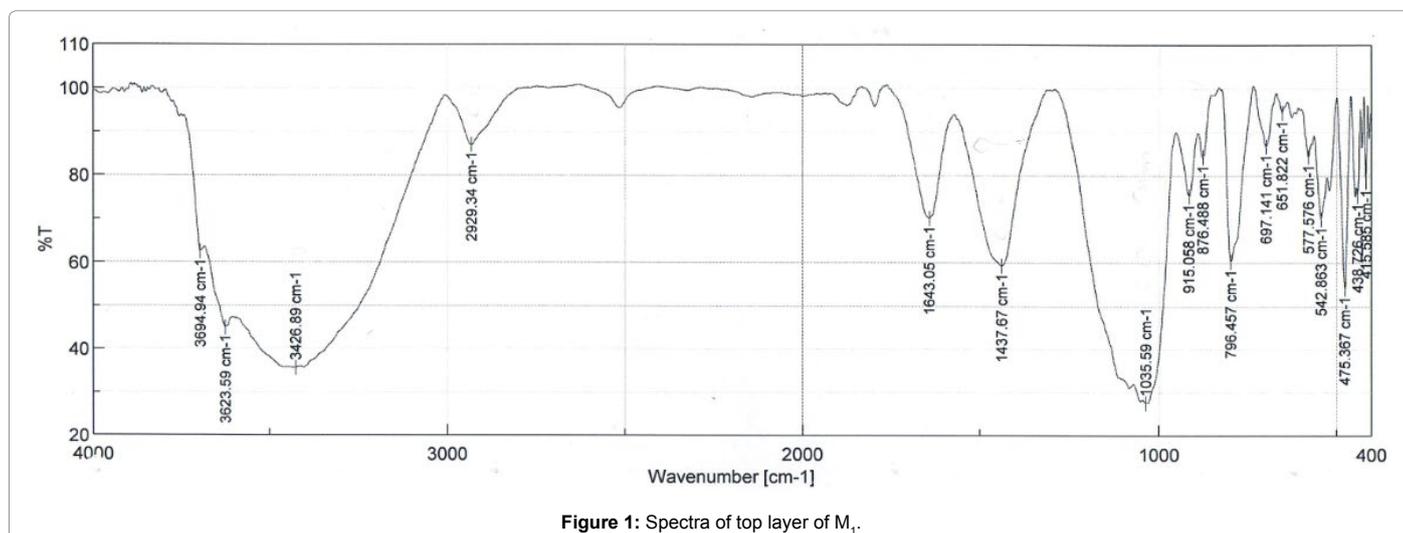
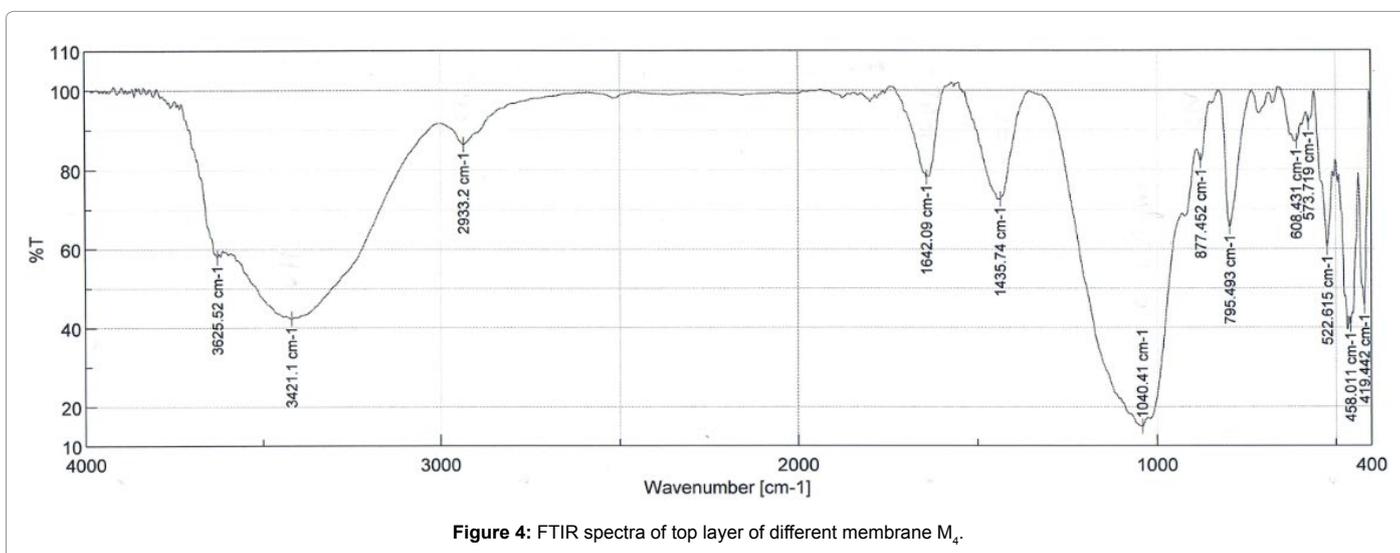
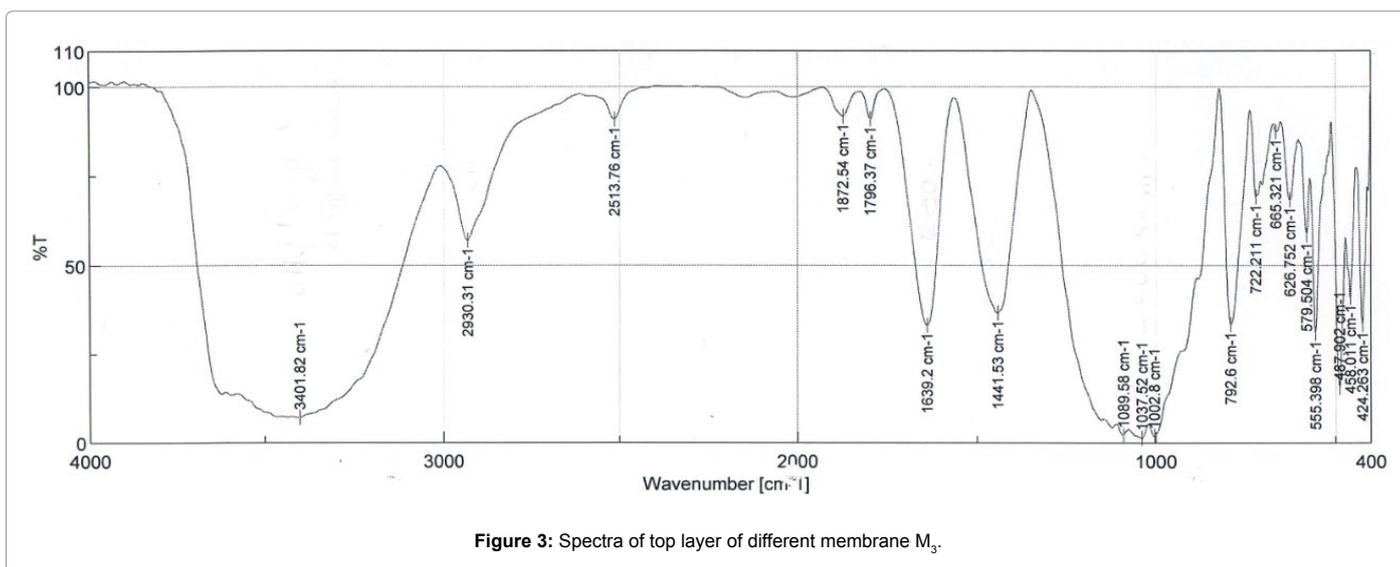
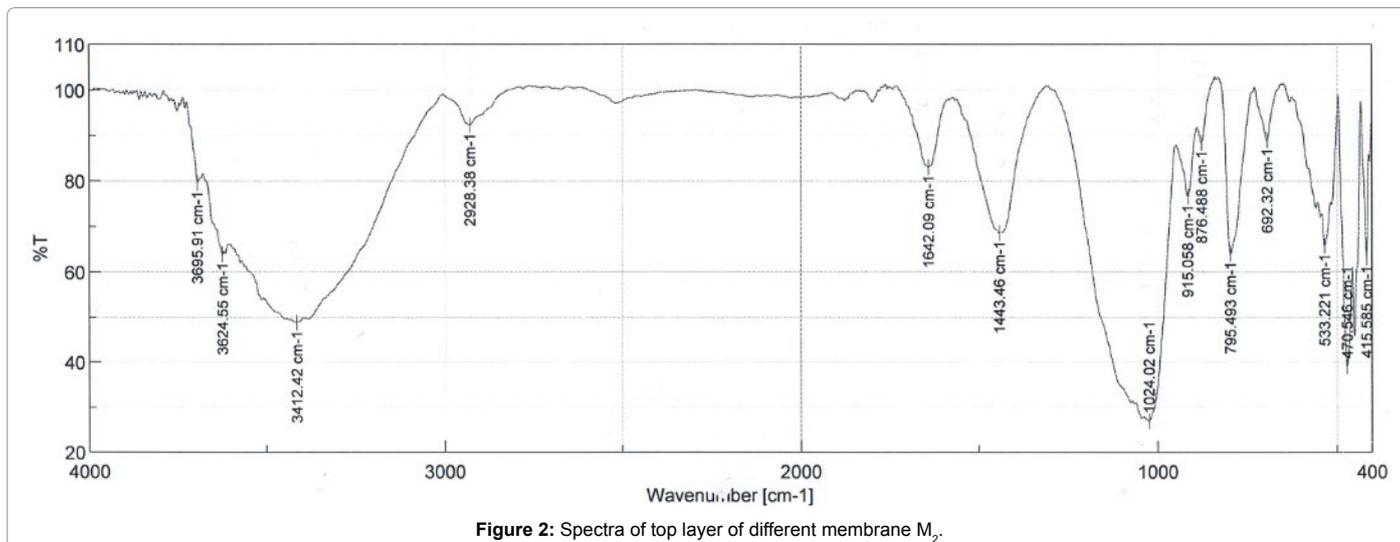


Figure 1: Spectra of top layer of  $M_1$ .



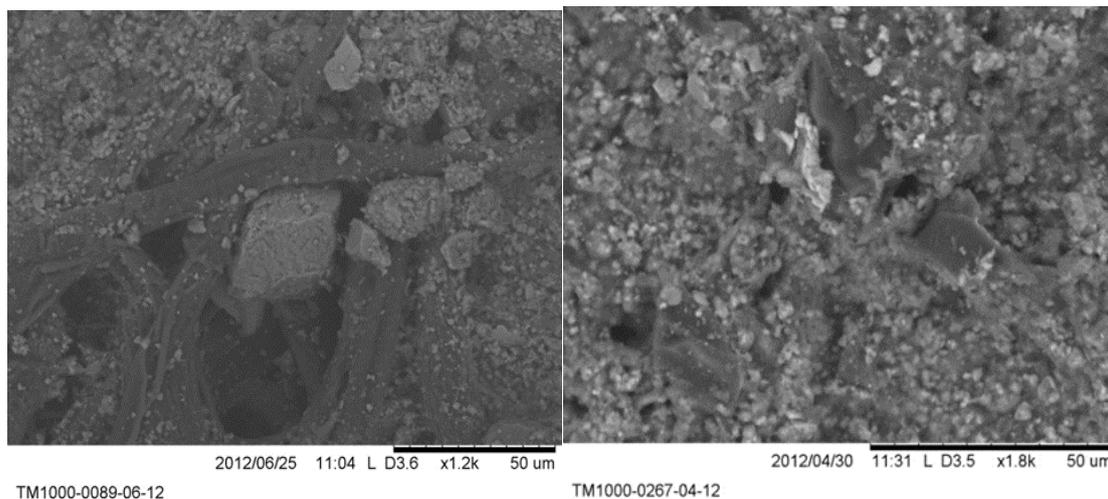


Figure 5: SEM photographs for binary and ternary membranes: (a) M<sub>1</sub>, (b) M<sub>2</sub>.

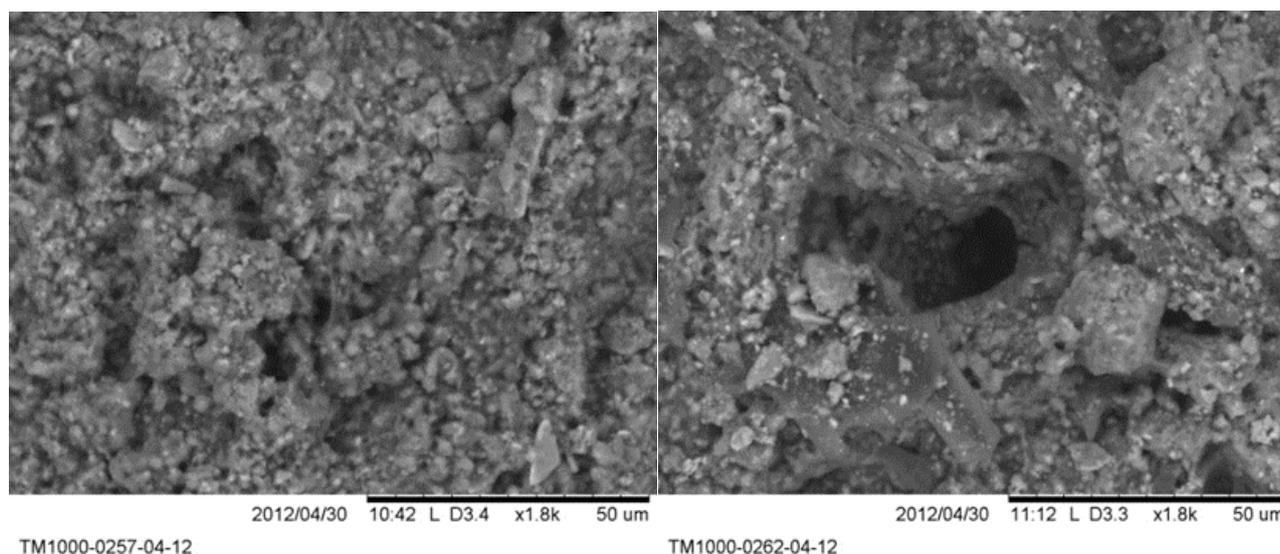


Figure 6: SEM photographs for binary and ternary membranes: (c) M<sub>3</sub>, (d) M<sub>4</sub>.

Selected Membrane	Amidon Concentration (g/L)	Bentonite concentration (g/L)	Activated carbon concentration (g/L)
M <sub>1</sub>	25	100	/
M <sub>2</sub>	25	100	12.5
M <sub>3</sub>	25	100	/
M <sub>4</sub>	25	100	12.5

M<sub>1</sub>, M<sub>2</sub>: Synthesized membranes with treated Bentonite; M<sub>3</sub>, M<sub>4</sub>: Synthesized membranes with natural Bentonite

Table 1: Different selected membranes.

Bacterial strains membranes	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
M <sub>1</sub>	-	-
M <sub>2</sub>	+	-
M <sub>3</sub>	+	+
M <sub>4</sub>	-	-

(+) Presence of bacteria in the filtrate; (-) Absence of bacteria in the filtrate.

Table 2: Results of bacteria filtration.

Fungal strains membranes	<i>Aspergillus niger</i>	<i>Penicillium sp</i>
M <sub>1</sub>	+	+
M <sub>2</sub>	+	+
M <sub>3</sub>	-	-
M <sub>4</sub>	-	+

(+) Presence of bacteria in the filtrate; (-) Absence of bacteria in the filtrate

Table 3: Results of the *A. niger* and *P. notatum* filtration.

Tested microorganisms	Selective membranes
<i>Escherichia coli</i>	M <sub>1</sub> , M <sub>4</sub>
<i>Pseudomonas aeruginosa</i>	M <sub>1</sub> , M <sub>2</sub> , M <sub>4</sub>
<i>Aspergillus niger</i>	M <sub>3</sub> , M <sub>4</sub>
<i>Penicillium notatum</i>	M <sub>3</sub>

Table 4: The retained selective membranes.

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