MILLIPORE

Effect of Membrane Filter Pore Size on Microbial Recovery and Colony Morphology

Summary

Membrane filters with a 0.45 µm pore size have long been recognized as the standard for growth of microorganisms. However, there is little published literature comparing the effects of different pore sizes on colony size and recovery.

The 0.45 µm pore size is used to recover bacteria and other microorganisms from many samples and environments—almost to the exclusion of other pore sizes. Only rarely are other sizes used for growth and recovery and there is little information available on the effects of different pore sizes on microorganisms. However, other pore sizes are commercially available for microbial enumeration and users will occasionally substitute a filter with a pore size larger or smaller than 0.45 µm in an attempt to improve their results.

This study takes a broad look at the influence of different pore sizes on some common microorganisms. It provides data on the effect of pore size on growth and recovery. The study compares a variety of microorganism/media combinations on a range of pore sizes: 0.22, 0.45, 0.7, 0.8, and 1.2 µm. The filtration method used in the study was a standard glass funnel and base with vacuum. Test filters were plated on solid (agar) media and compared against spread plates.

There was no universal pattern of results. Some microorganisms, such as *Micrococcus luteus* and *Candida albicans* showed no significant difference in recovery or colony size with membrane pore size. Other organisms such as *Pantoea agglomerans* showed no difference in colony size but had low recoveries on 1.2 and 0.22 µm membranes.

Acceptable recovery for membrane filters was defined as being ≥ 90% versus the controls (spread plates).* The 0.45 µm membranes met this definition with all test systems. Some test systems showed equivalent recoveries with other pore sizes but in no case were the results significantly better. The lowest recoveries were seen with extremes of the pore size range (1.2 and 0.22 µm).

Materials and Methods

The pore sizes used in this study were selected from the various sizes of mixed esters of cellulose membranes manufactured by Millipore (Table 1).

The microorganisms and media combinations used in this study were chosen as a broad representation of common membrane filter applications: pharmaceutical, food and beverage, USP testing, water testing, and general microbiology (Table 2).

As an adjunct to the recovery and colony size experiments, the test filters were tested for their retentive capabilities under the conditions of average use. Each filter was challenged with a low level of *Brevundimonas (Pseudomonas) diminuta* (the standard organism for retention testing) and the filtrate was retained for enumeration.

Table 1. Test Filters

Pore Size (µm)	Millipore filter code	Flow Rate (sec/500 mL)	Bubble Point (psi)	Typical Applications
0.22	GSWP	40 to 60	50 to 60	Sterile filtration
0.45	HAWG	30 to 50	30 to 36	Microbial testing of water, beverages, and general microbiology
0.7	HCWG	15 to 23	19 to 23	Fecal coliform testing in surface and wastewater
0.8	AAWG	10 to 16	14 to 18	Yeast and mold testing in beverages
1.2	RAWG	7 to 11	11 to 13	Yeast and mold testing in "hard-to-filter" beverages

Table 2. Test Systems

Microorganism	Source	Media	Temp. (°C)	Time (hours)
Primary effluent	Wastewater	m-Endo LES	35	24
Primary effluent	Wastewater	m-FC	44.5	24
Primary effluent	Wastewater	m-TEC	35 – 44.5	24
Bacillus subtilis	ATCC 13933	Tryptic soy agar	35	24
*Brevundimonas** *diminuta*	ATCC 19146	Tryptic soy agar	30	48
Candida albicans	ATCC 10231	Tryptic soy agar	35	24
*Clostridium sporogenes***	ATCC 11437	Tryptic soy agar	35	48
Enterobacter aerogenes	ATCC 49701	Tryptic soy agar	35	24
Escherichia coli	ATCC 25922	m-FC	44.5	24
Micrococcus luteus	ATCC 9341	Tryptic soy agar	35	24
Pantoea agglomerans	Well water	m-Endo LES	35	48
Pantoea agglomerans	Well water	Tryptic soy agar	35	24

*Previously grouped in the genera Pseudomonas

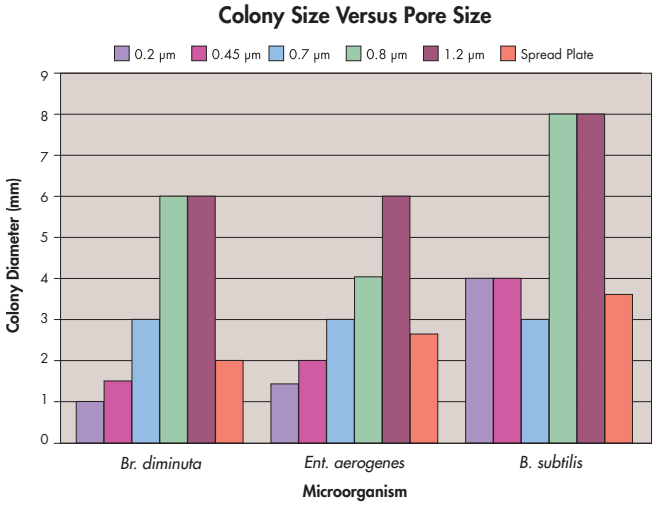
**Grown anaerobically using a Gas Pak jar (BBL)

Results

The selection of test systems was not intended to be exhaustive but to give a broad overview of microbial recovery in relation to filter pore size. Six different filter pore sizes were tested with 12 microorganism/media combinations that are representative of the types of microorganisms encountered by those using the membrane filter (MF) technique.

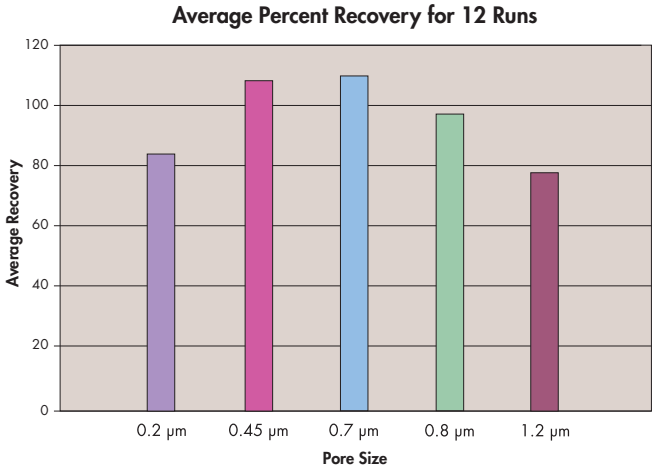
Colony Size

- Three test systems, *Br. diminuta, Ent. aerogenes, and B. subtilis,* showed differences in colony size with pore size.
  - Colonies grown on 1.2 and 0.8 µm filters were larger than colonies grown on other filter pore sizes or spread plates.
  - Colonies grown on 0.7 µm filters were the same size as, or slightly larger than, colonies grown on spread plates.
  - Colonies on 0.45 and 0.2 µm filters were the same size as, or some what smaller than, colonies grown on spread plates.
- Other test systems showed virtually no difference in colony size with any of the other pore sizes as compared to colonies grown on spread plates.



Microbial Recovery

- 0.45 µm and 0.7 µm filters demonstrated acceptable* recovery (≥ 90% versus spread plates) for all 12 test systems
- Although the average recovery for 0.8 µm filters was acceptable over the 12 test systems, the pore size had lower recoveries than 0.45 and 0.7 µm filters.
- Although 0.22 and 1.2 µm filters gave acceptable recoveries with some systems, their average recovery was significantly lower overall.

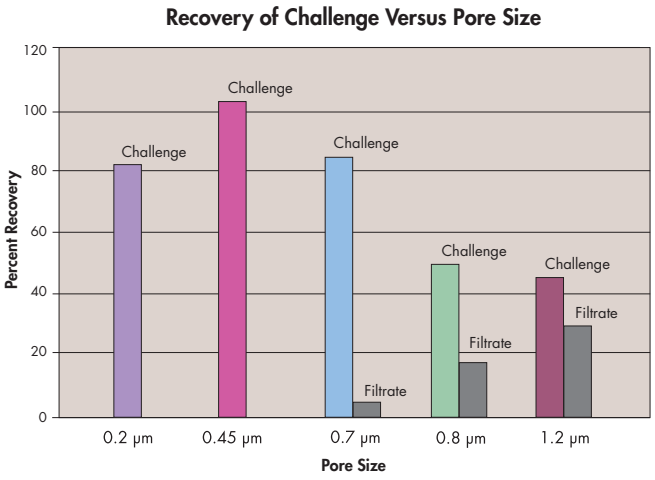


The average performance of each pore size was determined using all the test systems

*Standard Methods defines acceptable recovery on membrane filters as 90% or greater compared to spread plates.

Retention

- The larger pore sizes (1.2 and 0.8 µm) allowed significant passage of a small organism at low challenge levels (starved *Br. diminuta*) but there was no passage with the 0.45 or 0.22 µm pore sizes.
- Although 0.22 µm filters retained the challenge, the average recovery across all test systems was lower than 0.45 µm filters.
- Passage might be one reason why larger pore sizes (> 0.7 µm) showed lower recoveries than smaller pore sizes.



Overall

Recovery is much more complex than the retention of microorganisms on the surface of a membrane filter and the influence of pore size. It is a combination of factors that may include:

- The microorganism species and its condition—each microorganism has the potential to react differently
- The sieving effects of the pore size as it relates to the retention of specific microorganisms
- Type of medium and selectivity
- Structure and chemistry of the membrane filter
- Environmental conditions (e.g., moisture, incubation, temperature)

The effect of filter pore size on any specific microorganism/medium combination is not always predictable. If pore sizes other than those indicated by industry standards are used, they should be validated on relevant samples and media and compared to 0.45 µm.

This study confirmed that the standard 0.45 µm pore size is the most appropriate for general microbiological purposes. The 0.45 µm filters gave the most consistent recoveries across a variety of test systems and did not allow passage of the standard 0.2 µm sterilizing filter challenge microorganism, *B. diminuta,* under typical filtration conditions.

Conclusion

A membrane pore size larger than 0.45 µm can increase flow rate, throughput, and, occasionally, colony size (which makes the colonies easier to count). However, these larger pore sizes may not have sufficient retention for some microorganisms. Therefore, they are not well suited for total count applications.

Larger pore sizes can be used for enumerating specific organisms, such as fecal coliforms or yeast. They can also be used for difficult-to-filter samples where improved throughput or larger sample volumes are needed. In both cases, the filter's retention performance should be documented for the target microorganism(s).

Pore sizes smaller than 0.45 µm have the disadvantage of decreased flow rate, throughput, and, potentially, recovery. Therefore, the greater retentive properties of the 0.2 µm pore size have little benefit for the enumeration of bacteria, yeast, and molds in the variety of liquids considered in this study.

References

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater,* 20th Edition. American Public Health Association. 1015 Fifteenth Street, NW, Washington, DC. Part 9000.

Health Industry Manufacturers Association. *Microbiological evaluation of filters for sterilizing liquids*. HIMA Document No. 3, Vol. 4, April 1982. 34 pp.

J. Carter, "Evaluation of Recovery Filters for Use in Bacterial Retention Testing of Sterilizing-Grade Filters," PDA Jrnl. Of Pharm. Sci. and Technol. 50, 3, pp. 147 – 153 (1996).

To Place an Order or Receive Technical Assistance

MILLIPORE

For additional information call your nearest Millipore office:
In the U.S. and Canada, call toll-free 1-800-MILLIPORE (1-800-645-5476)
In the U.S., Canada and Puerto Rico, fax orders to 1-800-MILLIFX (1-800-645-5439)
On the Internet http://www.millipore.com
E-mail: tech_service@millipore.com

Millipore Worldwide

AUSTRALIA
Millipore Australia Pty. Ltd.
9A Byfield Street
North Ryde
NSW 2113
Tel. 1 800 222 111 (toll free)
or (02) 9888 8999
Fax (02) 9878 0788

AUSTRIA
Millipore Ges.m.b.H.
Hietzinger Hauptstrasse 145
A-1130 Wien
Tel. (01) 877-8926
Fax (01) 877-1654

BALTIC COUNTRIES
Millipore Oy
Ruukinkuja 4
FIN-02320 Espoo, Finland
Tel. +358 9 804 5110
Fax +358 9 256 5660

BELGIUM AND LUXEMBOURG
Millipore S.A.-N.V.
Rue de la Fusée 60
Raketstraat 60
B-1130 Brussels
Tel. (02) 726 88 40
Fax (02) 726 98 84

BRAZIL
Millipore Indústria e Comércio Ltda.
Rua Prof. Campos de Oliveira, 430
CEP 04675-100
Saõ Paulo-SP
Tel. (011) 5548-7011
Fax (011) 5548-7923

CANADA
Analytical Division
Millipore (Canada) Ltd.
36 Antares Drive, Suite 280
Nepean, Ontario
K2E 7W5
Tel. 1-800-645-5476
Fax 1-800-645-5439

BioProcess Division
Millipore (Canada) Ltd.
109 Woodbine Downs Blvd.
Unit 5
Etobicoke, Ontario
M9W 6YI
Tel. 1-800-645-5476
Fax 1-800-645-5439

Laboratory Water Division
Millipore (Canada) Ltd.
19 Thorne St. Suite 302
Cambridge, Ontario
N1R 1S3
Tel. 1-800-645-5476
Fax 1-800-645-5439

CHINA, PEOPLE'S REPUBLIC OF
Millipore China Ltd.
Suite 1209, 12/F
China Resources Building
No. 8 Jianguomenbei Avenue
Beijing 100005
Tel. (8610) 8519-1250
(8610) 8519-1058
Fax (8610) 8519-1255

Millipore China Ltd.
Room 1008, 10/F Metro Plaza
183 Tian He Road (N)
Guangzhou 510620
Tel. (8620) 8755-4021
Fax (8620) 8755-4350

Millipore Asia/China Ltd.
Suite 3201, Central Plaza
18 Harbour Road, Wanchai
Hong Kong
Tel. (852) 2803-9111
Fax (852) 2513-0313

Millipore China Ltd.
Suite 901 Hong Kong Plaza (S)
283 Huai Hai Road (M)
Shanghai 200021
Tel. (8621) 5306-9100
Fax (8621) 5306-0838

CZECH REPUBLIC
Millipore spol. s.r.o.
Ricanova 21
16900 Praha 6
Tel. 02-2051 3841
02-2051 3842
Fax 02-2051 4298

DENMARK
Millipore A/S
Odinsvej 9-19, st.
2600 Glostrup
Tel. 70 10 00 23
Fax 70 10 13 14

EASTERN EUROPE, C.I.S., AFRICA, MIDDLE EAST AND GULF
Analytical Division
Millipore S.A.
BP 116
67124 Molsheim Cedex
France
Tel. (33) 3.88.38.9536
Fax (33) 3.88.38.9539

BioProcess Division
Millipore Ges.m.b.H.
Hietzinger Hauptstrasse 145
A-1130 Wien, Austria
Tel. (43) 1 877-8926
Fax (43) 1 877-1654

Laboratory Water Division
Millipore S.A.
BP 307
78054 Saint-Quentin
Yvelines Cedex, France
Tel. (33) 1.30.12.70.00
Fax (33) 1.30.12.71.80

FINLAND
Millipore Oy
Ruukinkuja 4
02320 Espoo
Tel. (09) 804 5110
Fax (09) 256 5660

FRANCE
Millipore S.A.
BP 307
78054 Saint-Quentin
Yvelines Cedex
Tel. (01) 30.12.70.00
Fax (01) 30.12.71.80

GERMANY
Millipore GmbH
Hauptstraße 87
65760 Eschborn
Tel. (06196) 494-0
Fax (06196) 43901

HUNGARY
Millipore Kft.
Andor u. 47-49
1119 Budapest
Tel. 1-205 9784
Fax 1-205 9792

INDIA
Millipore (India) Pvt. Ltd.
50A 2nd Phase Ring Road
Peenya
560 058 Bangalore
Tel. (91) 80-839 46 57
Fax (91) 80-839 63 45

ITALY
Millipore S.p.A.
Via XI Febbraio, 99
20090 Vimodrone (Milano)
Tel. (02) 25.07.81
Fax (02) 26.50.324

Millipore S.p.A.
Via D. Sansotta 100
00144 Roma
Tel. (06) 52.03.600
Fax (06) 52.95.735

JAPAN
Nihon Millipore Ltd.
Mita Kokusai Bldg.
4-28, Mita 1-Chome
Minato-ku
Tokyo 108
Tel. (03) 5442-9711
Fax (03) 5442-
9736 Analytical
9737 BioProcess
9734 Lab Water

KOREA
Millipore Korea Co. Ltd.
Suite 711
City Air Terminal Building
159-6, Samsung-Dong
Kangnam-ku
Seoul 135-728
Tel. (822) 551-0990
Fax (822) 551-0228

MALAYSIA
Millipore Asia Ltd.
Suite 3.03 Wisma KT
No. 14 Jalan 19/1
46300 Petaling Jaya
Selangor
Tel. 603-7571322
Fax 603-7571711

MEXICO
Millipore S.A. de C.V.
Ings. Militares 85-PB
Col. Argentina Poniente
11230 México, D.F.
Tel. (525) 576-9688
Fax (525) 576-8706

THE NETHERLANDS
Millipore B.V.
Penningweg 33
Postbus 166
4870 AD Etten-Leur (N.B.)
Tel. 076-5022000
Fax 076-5022436

NORWAY
Millipore AS
Postboks 214 – Manglerud
0612 Oslo
Tel. 22 67 82 53
Fax 22 66 04 60

POLAND
Millipore Sp.z.o.o.
ul. Jasnodworska 7
01745 Warszawa
Tel. 22-669 12 25
22-663 70 31
Fax 22-663 70 33

PUERTO RICO
Millipore Cidra Inc.
P.O. Box 11977
Cidra, P.R. 00739-1977
Tel. (787) 273-8495
Fax (787) 747-6553

SINGAPORE
Millipore Singapore Pte Ltd.
31 Kaki Bukit Road 3
#06-08/11 Techlink
Singapore 417818
Tel. (65) 842 1822
Fax (65) 842 4988

SPAIN AND PORTUGAL
Millipore Ibérica, S.A.
Avda. Llano Castellano,13-3°
28034 Madrid
Tel. 917 283 960
Fax 917 292 909

Millipore Ibérica, S.A.
Balmes, 89-91, 8°
08008 Barcelona
Tel. 934 525 530
Fax 934 516 048

SWEDEN
Millipore AB
Box 7090
174 07 Sundbyberg
Tel. 08-628 6960
Fax 08-628 6457

SWITZERLAND
Millipore AG
Chriesbaumstrasse 6
8604 Volketswil
Tel. (01) 908-30-60
Fax (01) 908-30-80

Millipore S.A.
43, Chemin de Maillefer
1052 Le Mont-sur-Lausanne
Tel. (021) 641 2550
Fax (021) 641 2551

TAIWAN
Millipore Asia Ltd.
Unit #707, 7th fl
136 Sec.3 Jen-Ai Road
Taipei 10628
Tel. (886-2) 700-1742
Fax (886-2) 755-3267

Millipore Asia Ltd.
2F., No. 8, Lane 99
Pu-Ding Road
Hsin Chu City
Tel. (886-3) 571-0178
Fax (886-3) 572-9520

U.K. AND IRELAND
Millipore (U.K.) Ltd.
The Boulevard
Blackmoor Lane
Watford, Hertfordshire
WD1 8YW
Tel. (01923) 816375
Fax (01923) 818297

U.S.A.
Millipore Corporation
80 Ashby Road
Bedford, MA 01730-2271
Tel. (781) 533-6000
Fax (781) 533-3110

IN ALL OTHER COUNTRIES
Millipore Intertech
P.O. Box 255
Bedford, MA 01730 U.S.A.
Tel. +1 (781) 533-8622
Fax +1 (781) 533-8630

Lit. No.TB1025EN00 Printed in U.S.A. 12/00 00-301