-Supporting Information 2-

Batch and flow nitroaldol synthesis catalysed by *Granulicella tundricola* hydroxynitrile lyase immobilised on Celite R-633

José Coloma [1,2], Lidwien Teeuwisse [1], Muhammad Afendi [1], Peter-Leon Hagedoorn [1] and Ulf Hanefeld [1]*

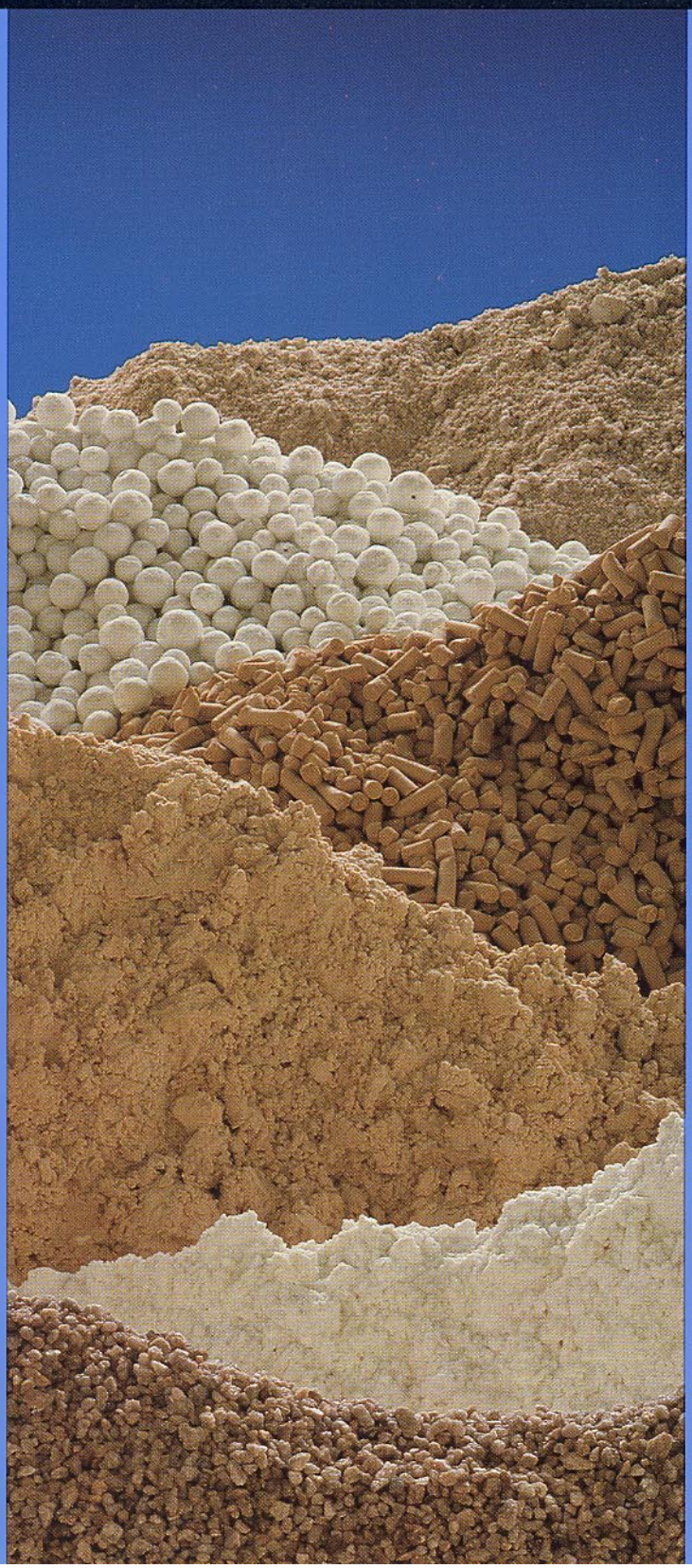
1 Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands; J.L.ColomaHurel@tudelft.nl (J.C); L.C.Teeuwisse@student.tudelft.nl (L.T.); MuhammadFarhanbinMuhammadAfendi@student.tudelft.nl (M.A); P.L.Hagedoorn@tudelft.nl (P.-L.H); u.hanefeld@tudelft.nl (U.H).
2 Universidad Laica Eloy Alfaro de Manabí, Avenida Circunvalación s/n, Manta 13-05-2732, Ecuador.

***Corresponding Author:** Ulf Hanefeld
Tel: +31(0)15-2789304; Fax: +31(0)15-2781415; Email: U.Hanefeld@tudelft.nl.
Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

Celite

Catalyst Carriers

For Biomolecule, Enzyme, and Cell Immobilization



Celite® Catalyst Carriers For Biomolecule, Enzyme, and Cell Immobilization

The immobilization of enzymes and microbes for commercial production allow their use in continuous processes rather than traditional batch reactors. Although biocarriers can and are used in batch and semi-batch systems, the more economical continuous systems have the advantages of improved utilization of both the feed stocks and enzymes or cells and better process control in come cases.

The carriers most commonly required for large scale use are made of glass or ceramic materials. Celite has developed a series of these carriers designed expressly for the biotechnology industry. Their manufacture is based on more than fifty years of experience with silica and silicates. Celite also has experience with various types of fiber glass and ceramic materials.

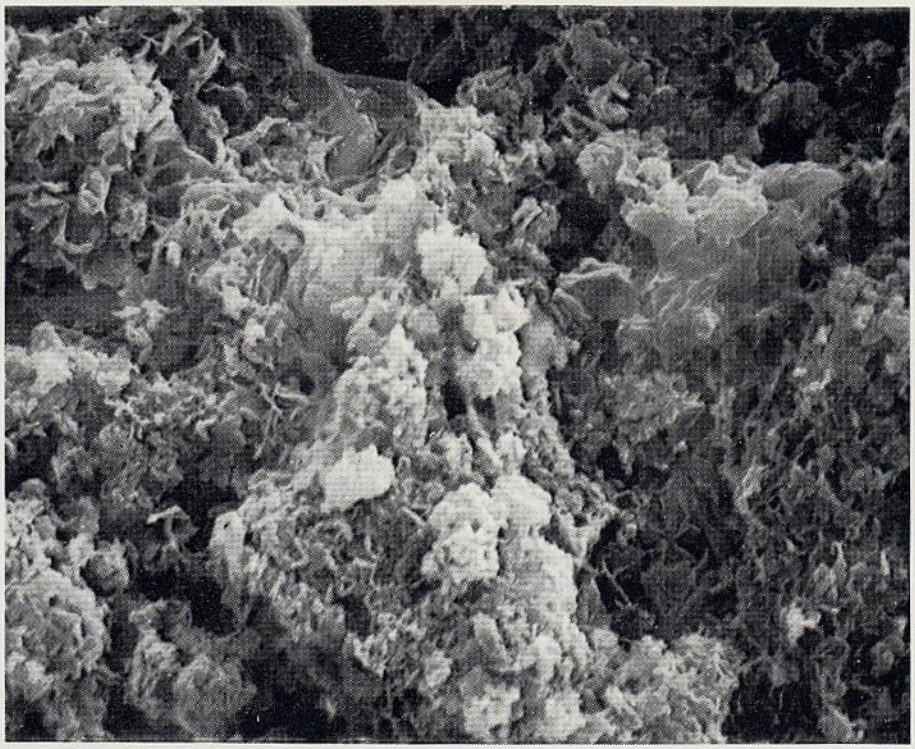
Immobilization requires closely-sized pores in the carriers. Properly-sized pores provide a number of advantages:

1. They maximize the concentration of the enzyme, biomolecule, or cell in the reactor.

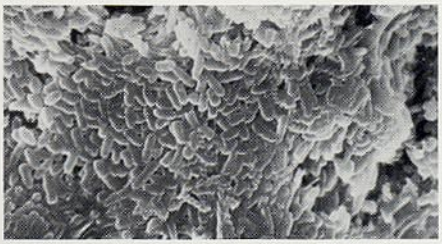
2. They protect the enzyme or cell from damaging fluid movement.

3. They help to protect the enzyme from destructive bacteria within the reactor system.

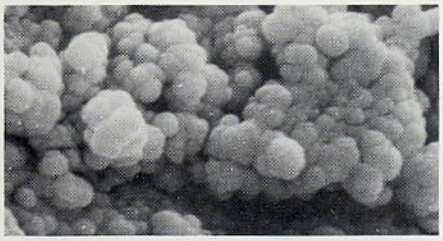
This series of catalyst carriers provides low-cost materials with controlled pore sizes. The low cost aspect makes many potential processes economical. Intended for use in pharmaceuticals, chemicals, waste disposal and many other applications, these carriers provide the combination of properties necessary for an efficient operation.



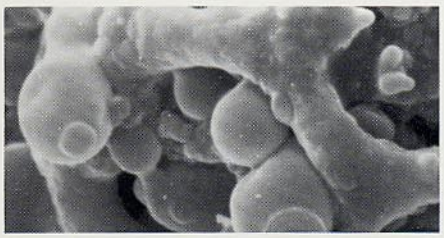
Scanning electron micrograph of typical enzyme carrier pore structure magnification.



Bacterial cells immobilized on Celite proprietary controlled porosity carrier.

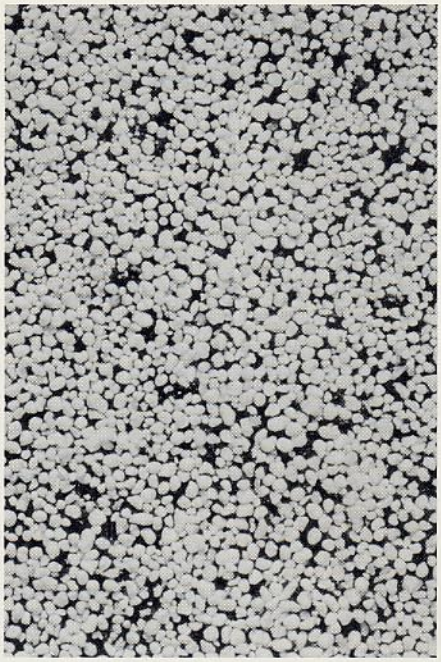


Hybridoma cells immobilized within and on Celite engineered porosity matrix.



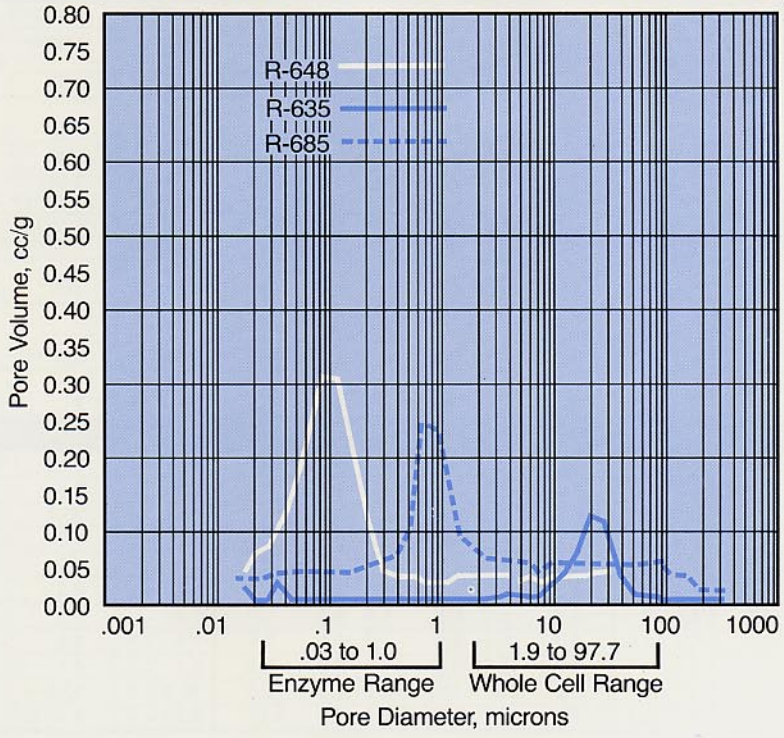
Yeast cells immobilized within Celite carrier for continuous processing.







Celite® Bio Catalyst Carrier Pore Size Distribution Curves*



*Mercury Intrustion Method (4 to 15,000 psi)

The carriers shown in this brochure are just an example of our capabilities. There are very few standard processes in the biotechnology field and, therefore, custom-made products for specific applications must be considered. Celite will work with individual customers in developing carriers best suited for specific applications. Information on alternatives in selecting pore sizes and volumes, carrier strength, sizes and shapes can be supplied.

Carrier Selection

Selecting a carrier involves defining the properties which are most important in the carrier. Typically, pore diameter and volume, strength, chemical inertness and carrier shape are most critical. These and other properties are interrelated and compromises can be made to produce the most economical carrier.

Pore Diameter

Production rates are greatly affected by the concentration of the enzymes or microbes and by the ease of diffusion to them. It has been found that by maximizing the concentration and accepting the resulting diffusion rates gives the best performance. The highest loadings are obtained when the pore diameters are based on the enzyme or microbe diameters. Pores which are one to ten times the size of the largest involved element typically provide the highest production rates.

In whole cell immobilization, the pore diameters are based on the major cell dimensions. Living systems require additional care to insure adequate space for cell reproduction. Typically, diameters of 1 to 50 microns are needed to accommodate the cells.

In enzyme processes, the desired pore diameter is based either on the enzyme's molecular weight or possibly on the dimensions of the reacting organic molecules. The desired diameters are usually between 200 and 2000 Angstroms.

Pore Volume

The pore volume is very important since it indicates the volume available for microbes to occupy. Specifically, only the volume of the pores within the useful size range should be considered. High pore volumes maximize the concentration per volume in the reactor. Although the pore volume can be controlled, there are trade-offs with other carrier properties, especially the carrier hardness.

Surface Area

The surface area is an indication of the number and shape of the carriers' pores and a measure of the available enzyme or molecular bonding sites. A high surface area is expected in a small pore carrier. The small pore carriers, such as Celite 640 and Celite 650, have surface areas of over 60 square meters per gram. The microbe carriers have relatively low surface areas but large pore diameters and volumes to allow better biomass accumulation.

Hardness

The mechanical strength of the carrier is determined by the composition, production process and physical size. The porosity of the carrier very signficantly affects its strength. Increases in pore volume have a large negative impact on particle strength. A minimum acceptable strength should be established for a given application, thereby allowing the optimization of the carrier's remaining properties.

Particle Size

Celite catalyst carriers can be produced in several forms: powder, pellet and sphere. The shape of these carriers can be custom fabricated in sizes ranging from 1.0 to 10 millimeters in diameter.

Diffusion limitations in reaction processes often require smaller particle sizes. The more commonly used carriers are in the range of 0.3 to 1.0 millimeters. However, powders can be produced in the micron range.

Chemical Composition

Celite catalyst carriers are made of rigid, inorganic materials. These materials provide the advantages of thermal and chemical stability, mechanical strength and rigidity and microbial resistance.

Diatomite, one of the basic raw materials, is compatible with a very wide range of aqueous and organic environments. It is currently used in the filtration of drugs, pharmaceuticals, beverages, acids and petrochemicals. Although each individual application must be considered separately, Celite catalyst carriers are recommended wherever high-silica glass is appropriate.

Typical physical properties and chemical analysis for our standard catalyst carriers are shown below.

These concepts are the basis for the selection of an immobilization carrier. They are to be considered as general guidelines in determining the parameters required for specific applications. Our technical staff can aid you in the selection of the most appropriate carrier.

Materials carriers can also be custom-tailored to meet your specific enzyme and microbe immobilization needs. Contact Celite Biochemistry Products & Services for additional information, samples and recommendations with regard to carrier requirements.

Typical Physical Properties

Product	R-600	R-625	R-630	R-631	R-632	R-633	R-634	R-635	R-640	R-646	R-647
Form	pellet (1/8")	pellet (1/8")	sphere (3/8)	sphere (8/14)	sphere (14/30)	sphere (30/50)	sphere (50/100)	pellet .25"D x .50"L	pellet (1/8")	sphere (8/14)	sphere (14/30)
Mean Pore Diameter, μ	0.38	5.5	6.6	6.0	7.0	6.5	5.0	20	0.03	0.04	0.07
Surface Area, B.E.T., m^2/g	19	12.4	1.3	1.0	2.0	1.3	2.1	0.27	61	45	51
Total Pore Volume, cc/g	0.938	0.74	1.45	1.45	1.19	1.47	1.08	0.61	0.76	0.8701	0.9723
Volume Fraction 400-1000 A, cc/g	0.072 (7.7%)	0.004 (0.5%)	0.008 (0.5%)	0.003 (0.2%)	0.006 (0.5%)	0.008 (0.5%)	0.005 (0.5%)		0.099 (13.0%)	0.1690 (19.4%)	0.1769 (18.2%)
Volume Fraction 1.0-50μ, cc/g	0.086 (9.2%)	0.669 (94.4%)	1.362 (94.0%)	1.322 (91.3%)	1.096 (92.0%)	1.424 (96.2%)	1.030 (95.4%)	0.513 (83.6%)	0.065 (8.6%)	0.1239 (14.2%)	0.2213 (22.8%)
Water Absorption, % by weight, pellet method	100.8	86	170	60	84	240	210	60	93	159	163
Crush Strength — Monsanto Hardness Test, kg	1.8	1.6	1.3	61-69†	15-24†	27-28†	0-5†	8	2.2	5†	4.7†
Bed Density (compacted) lbs/ft^3	26	32	21	17	20	22	24	32	29	25	24

Typical Chemical Analysis %

SiO_2	84.6	84.8	88.2	88.2	88.2	88.2	88.2	82.3	87.0	87	87
AL_2O_3	7.5	6.4	3.5	3.5	3.5	3.5	3.5	7.2	6.1	6.1	6.1
CaO	0.9	1.7	0.5	0.5	0.5	0.5	0.5	2.6	0.9	0.9	0.9
MgO	1.0	1.1	0.6	0.6	0.6	0.6	0.6	1.2	0.8	0.8	0.8
FE_2O_3	1.7	2.1	1.3	1.3	1.3	1.3	1.3	1.9	1.6	1.6	1.6
Na_2O	1.1	2.7	4.6	4.6	4.6	4.6	4.6	3.3	1.2	1.2	1.2
K_2O	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.9	0.4	0.4	0.4
P_2O_5	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.1	0.1	0.1
TiO_2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1
LOI	2.1	0.4	0.3	0.3	0.3	0.3	0.3	12.3	2.1	2.1	2.1
pH of slurry	9.4	10.0	9.8	9.5-10.5	9.5-10.5	10.4	9.5-10.5	8.0*	8.9	8.5-9.0	8.5-9.0

* Buffered slurry
†Hardness, % fines produced by 100 psi load

Typical Physical Properties

Product	R-648	R-649	R-650	R-660	R-670	R-675	R-680	R-681	R-682	R-685	R-690
Form	sphere (30/50)	sphere (50/100)	pellet (1/8")	pellet (1/8")	pellet (1/4")	pellet (1/8")	powder	powder	powder	powder	pellet (1/8")
Mean Pore Diameter, μ	0.14	0.25	0.05	0.05	0.4	0.05	1.8	4.0	1.8	2.0	0.19
Surface Area, B.E.T., m^2/g	46	39	88	116	25	71	105	2.5	5.6	175	85
Total Pore Volume, cc/g	1.186	0.7844	0.71	0.464	0.64	0.511	5.47	3.23	2.83	5.53	0.432
Volume Fraction 400-1000 A, cc/g	0.0234 (19.7%)	0.1654 (21.1%)	0.120 (16.8%)	0.057 (12.4%)	0.060 (9.4%)	0.338 (65.0%)	0.666 (12.1%)	0.003 (0.1%)	0.019 (0.7%)	0.45 (8.1%)	0.050 (11.6%)
Volume Fraction 1.0-50μ, cc/g	0.394 (33.2%)	0.1632 (20%)	0.049 (6.9%)	0.012 (2.6%)	0.002 (0.3%)	0.002 (0.3%)	3.023 (55.3%)	3.117 (96.6%)	2.347 (82.8%)	0.65 (11.8%)	0.024 (5.5%)
Water Absorption, % by weight, pellet method	160	160	80	63	62	64	480	250	190	Hydrophobic	62
Crush Strength — Monsanto Hardness Test, kg	4.1†	0.3†	3.5	6	7	7	N/A	N/A	N/A	N/A	2.3
Bed Density (compacted) lbs/ft^3	25	25	30	38	34	38	8	11	13	17	39

Typical Chemical Analysis %

SiO_2	87.0	87	85.1	63.9	85.1	59.9	57.3	93.6	92.9	63.9	54.0
AL_2O_3	6.1	6.1	7.0	3.1	7.5	6.4	2.9	2.3	3.2	3.1	35.0
CaO	0.9	0.9	0.9	0.4	0.8	16.3	25.7	0.2	0.3	0.4	0.6
MgO	0.8	0.8	0.9	14.8	0.9	1.0	0.7	0.5	0.6	14.8	0.9
FE_2O_3	1.6	1.6	1.7	1.5	1.7	1.4	0.8	0.8	1.1	1.5	1.5
Na_2O	1.2	1.2	1.3	0.1	0.9	0.7	0.2	2.3	0.5	0.1	0.5
K_2O	0.4	0.4	0.6	0.5	0.5	0.4	0.5	0.4	0.7	0.5	0.4
P_2O_5	0.1	0.1	0.2	0.2	0.1	0.5	0.2	0.1	0.2	0.2	0.1
TiO_2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
LOI	2.1	2.1	2.2	13.0	2.3	10.6	10.6	0.4	0.9	13	6.1
pH of slurry	8.5-9.0	8.5-9.0	7.9	9.9	7.4	9.7	8.8	10.1	6.6	7.4	8.5

* Buffered slurry
†Hardness, % fines produced by 100 psi load

The physical or chemical properties of Celite Corporation products represent typical, average values obtained in accordance with accepted test methods and are subject to normal manufacturing variations. They are supplied as a technical service and are subject to change without notice. Check the nearest Celite Corporation sales office to assure current information.

Headquarters
Celite Corporation
P.O. Box 519
Lompoc, CA 93438-0519
U.S.A.

For Export
Tel: 805-735-7791
Telex: 62776493 ESL UD
Fax: 805-735-5699

United States

Eastern
Celite Corporation
Wayne Interchange, Plaza II
155 Route 46
Wayne, NJ 07470
201-256-5435

Southern
Celite Corporation
P.O. Box 105545
Atlanta, GA 30348
404-263-5538

Midwestern
Celite Corporation
455 S. Frontage Rd., Unit 102
Burr Ridge, IL 60521
708-920-1880

Western
Celite Corporation
P.O. Box 519
Lompoc, CA 93438-0519
805-735-7791

Canada
Celite Canada Inc.
295 The West Mall
Etobicoke, Ontario M9C 4Z7
Tel: 416-626-8175
Fax: 416-626-8235

Europe
Celite France
9 rue du Colonel-de-Rochebrune
B.P. 240
92504 Rueil-Malmaison Cedex
France
Tel (1) 47 49 05 60
Telex: CELITE 631969 F
Fax: (1) 47 08 30 25

Celite (UK) Limited
Livingston Road
Hessle HU13 OEG
North Humberside
England
Tel: (482) 64 52 65
Telex: 592160
Fax: (482) 64 11 76

Celite Italiana s.r.l.
Viale Pasubio No. 6
20154 Milano, Italy
Tel: 654531
Telex: 311136 MANVI I
Fax: 39 2 29005439

México
Celite México S.A. de C.V.
Alejandro Dumas No. 103, 3er. piso
Col. Polanco
C.P. 11560 México, D.F.
Tel: 525 203-5611
Fax: 525 255-1835
México

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