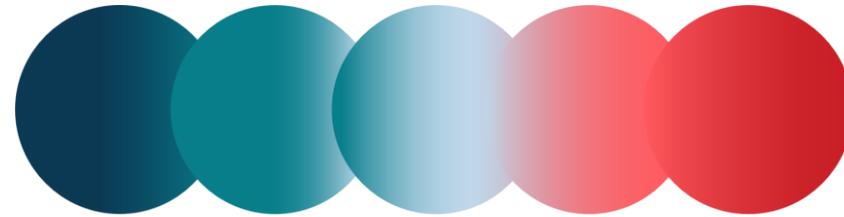




Funded by
the European Union



INTEGRANO

**MULTIDIMENSIONAL INTEGRATED QUANTITATIVE APPROACH TO ASSESS SAFETY AND
SUSTAINABILITY OF NANOMATERIALS IN REAL CASE LIFE CYCLE SCENARIOS USING
NANOSPECIFIC IMPACT CATEGORIES**

WP2

Experimental Data Generation: NMs provision and characterisation M-Measure (I)

12M Annual General Meeting

Turin - Italy
29-30 January 2025

Tasks

Task 2.1	Synthesis and Provision of the NM groups for targeted applications Leader: <u>CENTI (Lorena Coelho)</u> ; Partners: UNITO, CNR, BIU, AITEX	M3-M24
Task 2.2	Data mining: collecting info available on INTEGRANO target materials Leader: <u>CNR (Anna & Ilaria)</u> ; Partners: UNITO, CENTI, BIU, AITEX	M1-M18
Task 2.3	NMs Characterisation program for selected NMs: size, morphology, p-chem properties Leader: <u>CNR (Anna & Ilaria)</u> ; Partners: UNITO, CENTI, BIU, AITEX	M7-M36
Task 2.4	Characterisation and Detection of NMs and NEPs in real-case LC scenarios Leader: <u>CNR-ISAC (Alessia Nicosia)</u> ; Partners: PRJ, CENTI, UNITO, ARCHE, B4C, UniMIB, RoV, DRT, VERL	M13-M42
Task 2.5	Determination of safe condition of Use (CoU) and Risk Assessment (RA) Leader: <u>ARCHE (Joonas Koivisto)</u> ; Partners: CNR, UniMIB, JRC	M19-M42

Gantt

WP2			Year 1				Year 2				Year 3				Year 4			
Task	Title	Leader	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
2.1	Synthesis and Provision of the NM groups for targeted applications	CENTI																
2.2	Addressing case studies specific goal and scope	CNR																
2.3	NMs Characterisation program for selected NMs: size, morphology, p-chem properties	CNR																
2.4	Characterisation and Detection of NMs and NEPs in real-case LC scenarios	CNR																
2.5	Determination of safe condition of Use (CoU) and Risk Assessment (RA)	ARCHE																

Deliverables

Del.	Title	Lead Beneficiary	Diss. Level	Due Month	Date
D2.1	Set of NMs samples	CENTI (Lorena Coelho)	PU	24	December 2025
D2.2	INTEGRANO integrated database (DB) periodic release	CENTI (Lorena Coelho)	PU	36	December 2026
D2.3	DB on NMs detection campaigns in real and simulated environment for Fate Factors assessment	CNR (Alessia Nicosia)	PU	42	June 2027
D2.4	Report on Conditions of Safe Use	ARCHE (Joonas Koivisto)	PU	42	June 2027

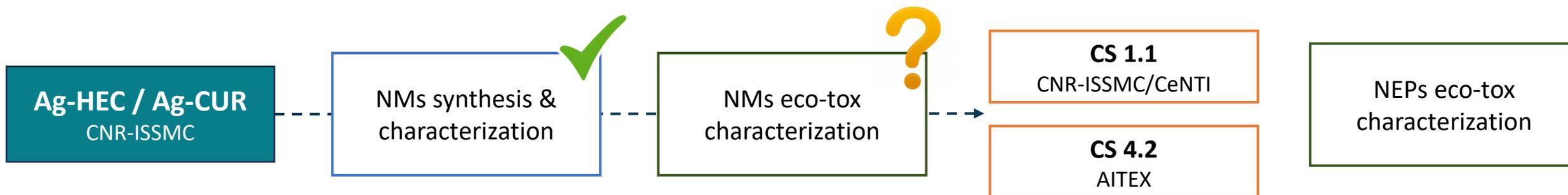
Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted

- Task 2.1 – Synthesis and Provision of the NM groups for targeted applications
- Task 2.2 – Data mining: collecting info available on INTEGRANO target materials
- **Task 2.3 – NMs Characterisation program for selected NMs: size, morphology, p-chem properties**

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



CS 1.1

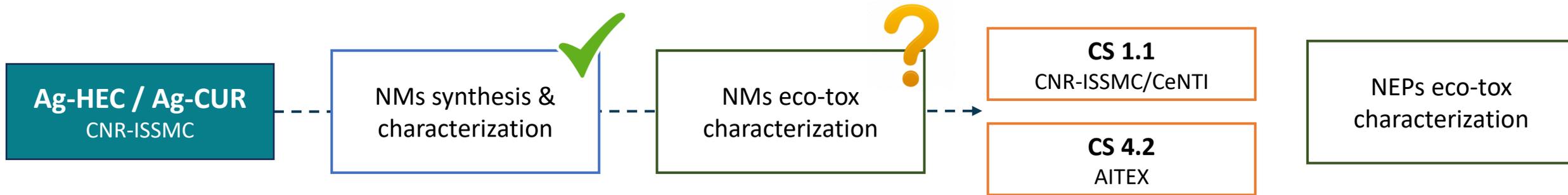
- AgHEC and AgCUR already optimized in previous projects;
- Coating on polyester 100% PES with 145 g/m², supplied by AITEK, with 3 concentrations (0.1, 0.05 and 0.01 wt.%)
- Higher concentration of Ag for AgHEC compare with AgCUR:
 - Necessary assess the Ag amount after 1 and 5 WC to verify the lowest Ag concentration ensuring a homogeneous stable coating.

Optimization goal:

- 1 KDF: Agnanosol concentration
- 3 KPIs: Ag loading, adhesion (washing fastness) and antibacterial

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted

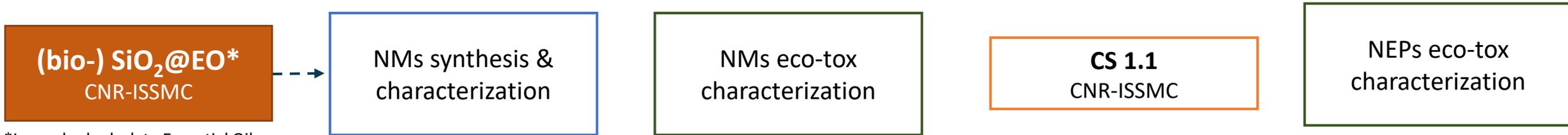


CS 4.2

- Two CA nanofiber membranes were produced by electrospun with around 550 nm and 0,34 and 4,15 g/m²;
- Comparing to a commercial FFP3, both membranes exhibit an exceptionally high-quality factor, indicating that the CA is a promising air filter that effectively removes particles while ensuring good breathability;
- Ag-HEC incorporation on CA nanofiber and characterization are ongoing.

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



*Lavander hydrolate Essential Oil

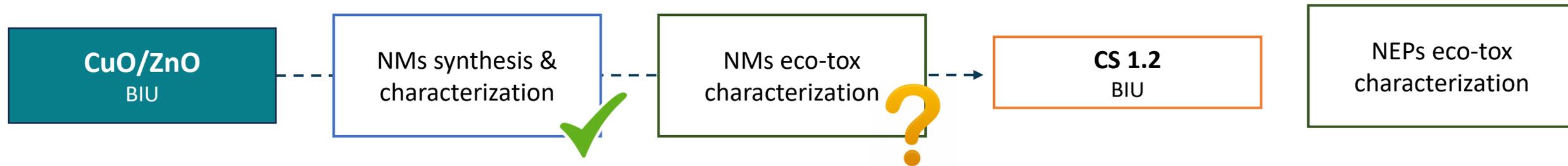
- CNR-ISSMC optimized the NF in previous project with SiO₂ by spray-drying and Stöber methods;
- Is under optimization the study with bio-SiO₂ by spray-drying and the material is under characterization:
 - TGA for EO quantification;
 - Antibacterial activity

Optimization goal:

- 1 KDF: active ingredient composition EO in water (later EO/water ratio) and SiO₂:EO ratio
- 3 KPIs: adsorption (loading) and desorption (release) and antibacterial activity.

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



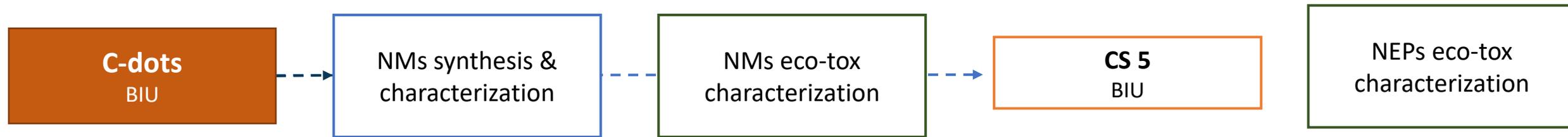
- Synthesis and incorporation on the same step;
- The coated textiles showed homogenous and dense coatings of metal oxides;
- **ZnO-coated textiles** with *E. coli* and *S. aureus* bacteria resulted in a **more than 4-log reduction in planktonic and biofilm bacterial growth mode**;
- **CuO-coated textiles** completely inhibited the growth of *S. aureus* biofilm bacteria and **significantly reduced (5-log) the planktonic growth**.

Optimization goal:

- 32 samples (CuO + cotton/polyester-cotton, ZnO + cotton/polyester-cotton) will be prepared following a DoE matrix for subsequent leaching investigations
- KDF1: precursor concentration
- KDF2: reaction time

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



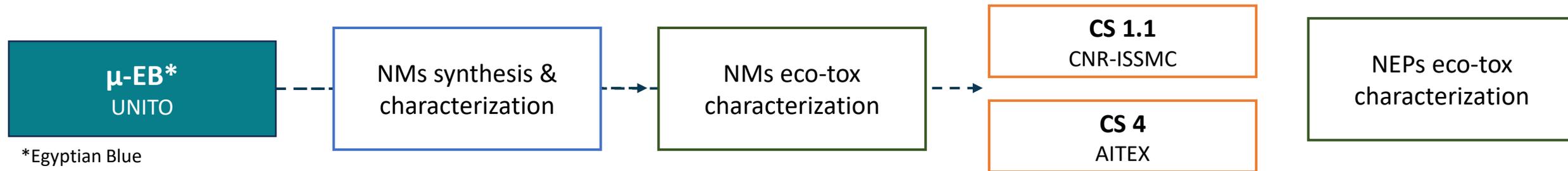
- Fluorescent C-dots were synthesized using Olive, Rosemary, Thyme, and Salvia Leaves as precursors;
- The properties of synthesized C-dots were characterized by TEM (size in the range of **3-5 nm**), and FL (~**450 nm**, the color of synthesized CDs' aqueous solution under daylight is yellow and blue under UV light), and antibacterial activity.
- Olive and Rosemary C-dots with MIC against *S. aureus* of **0,625 mg/ml** were chosen for further coating.

Optimization goal of the coating procedure with **two KDFs**:

- KDF1: precursor concentration
- KDF2: reaction time

Tasks 2.1 – 2.3

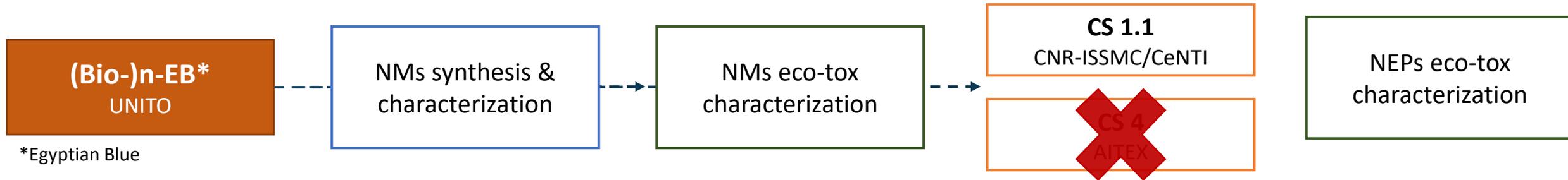
Overview of the NMs and NEPs targeted



- So far, from the 6 samples produced and characterized, there was no significant difference:
 - The sedimentation is very fast (diameter around 10 μm), which will limit its application in NEPs by some of technologies (spray coating);
 - Compound **I2B and I3A** showed the best performance in **inhibiting the growth of *E. coli* (5 and 10 mg/mL)**, but none of the compounds showed bactericidal activity at the concentration evaluated. Against *S. aureus*, all compounds were ineffective;
- CNR-ISSMC is studying the size reduction by ball milling - may affect luminescence effect
- According to the results, consider other options for exfoliation: hydrothermal, ultrasound or microwave process
- It was not observed sufficient performance to validate its effectiveness as an air filtering device – **will not be used in CS4**
- Bio-SiO₂ already sent for testing.

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



Optimization goal - exfoliation:

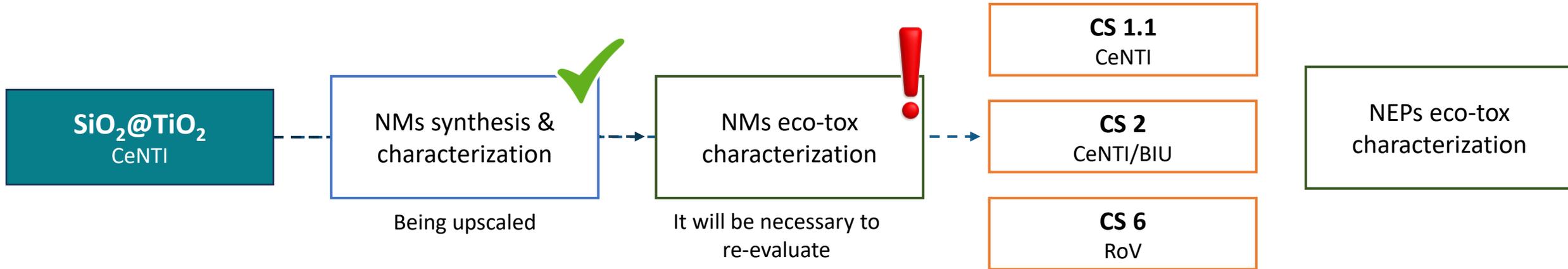
- 2 KDF: time and milling
- 3 KPIs: luminescence

Optimization goal – dip-coating:

- 1 KDF: EB concentration
- 4 KPI: EB loading, washing fastness, luminescence, antibacterial properties.

Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



CS 1.1

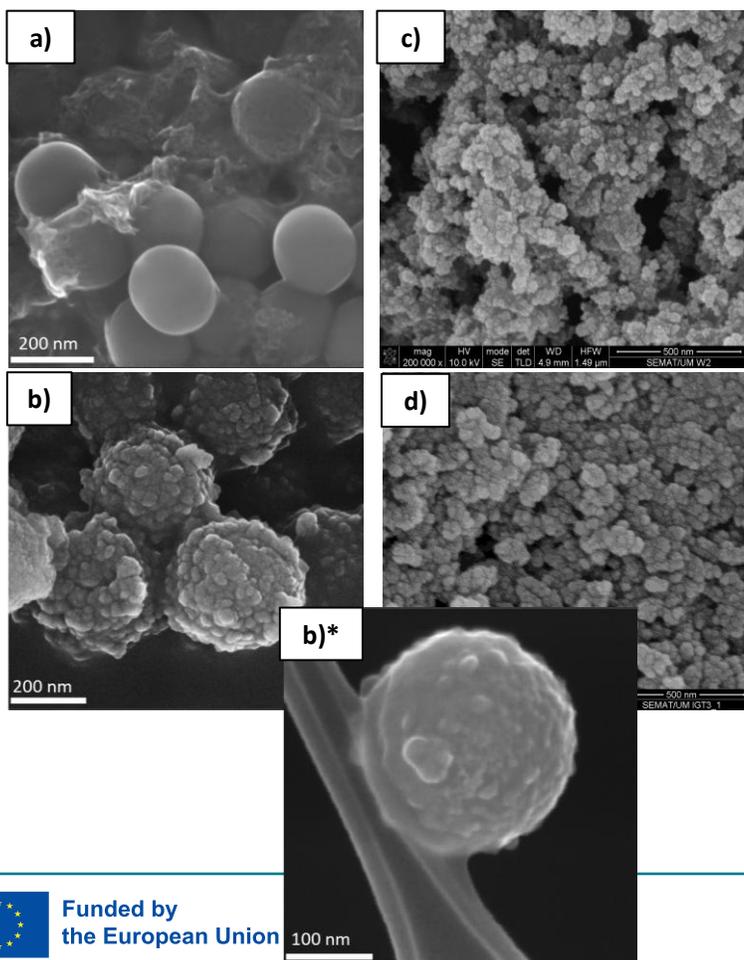
- Spray coating at CeNTI: for low amount applied with stand alone unit, later upscale with the pilot plant (not fully yet installed);
- Finalise KDFs for incorporation step

CS 2

- Spray coating at CeNTI (waiting for the membranes to be sent by B4C)
- Sonochemical coating at BIU

(Bio)-SiO₂@TiO₂ – Case Studies

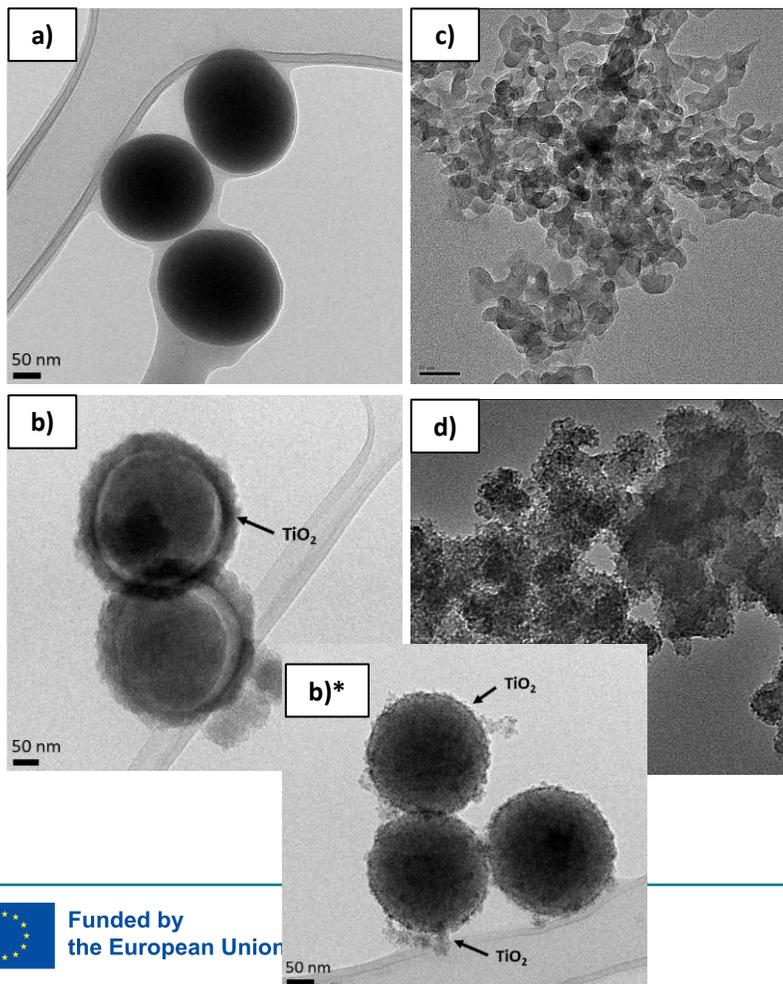
SEM



Sample	Size (nm)	Si %wt	Ti %wt	O %wt
a) NF • SiO ₂ • NP • 1	185 ± 11	9.9 ± 0.1	-	68.0 ± 0.2
b) NF • SiO ₂ @TiO ₂ HT • NP • 1	284 ± 24	8.1 ± 0.2	11.2 ± 2.4	29.5 ± 0.8
b)* NF • SiO ₂ @TiO ₂ CALC • NP • 1	211 ± 33	0.7 ± 0.0	0.5 ± 0.1	72.1 ± 0.5
c) NF • Bio-SiO ₂ • NP • 1	26 ± 9 37.2 ± 6.4	37.4 ± 0.2	-	57.6 ± 0.2
d) NF • Bio-SiO ₂ @TiO ₂ HT • NP • 1	40.8 ± 8.7	27.8 ± 0.3	11.7 ± 0.4	59.4 ± 0.4

(Bio)-SiO₂@TiO₂ – Case Studies

TEM

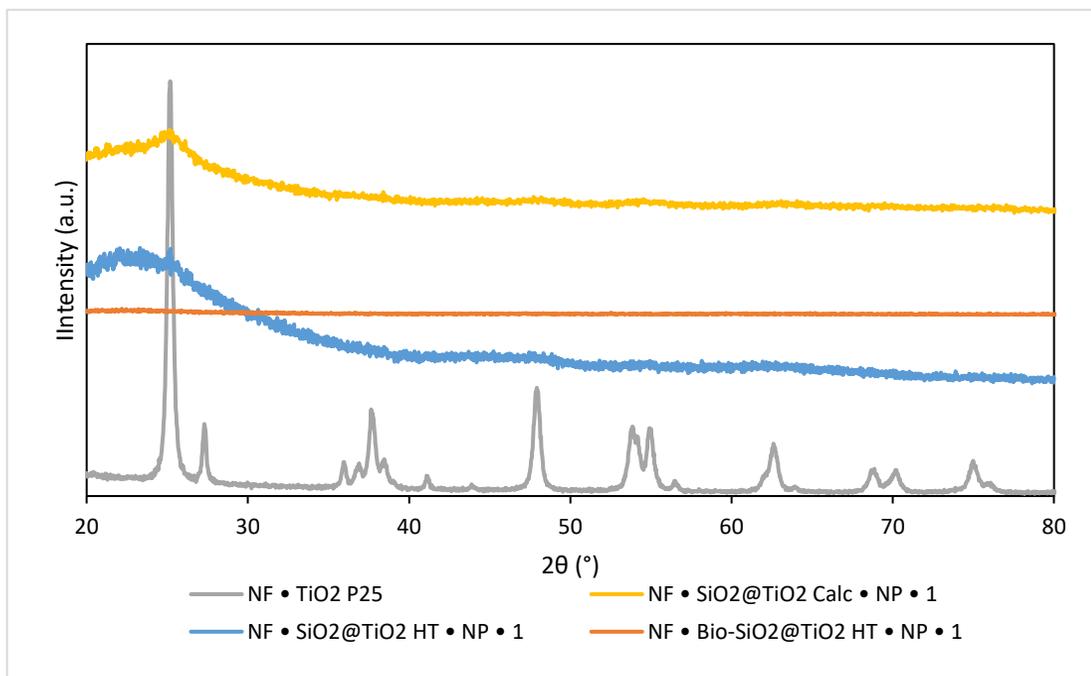


BET

Sample	BET SSA (m ² /g)	V micropore (cm ³ /g)	V meso/macropore (cm ³ /g)	V total (cm ³ /g)
a) NF • SiO ₂ • NP • 1	18	-	0.04	0.04
b) NF • SiO ₂ @TiO ₂ HT • NP • 1	280	0.04	0.14	0.18
b)* NF • SiO ₂ @TiO ₂ CALC • NP • 1	52	0.01	0.07	0.08
c) NF • Bio-SiO ₂ • NP • 1				
d) NF • Bio-SiO ₂ @TiO ₂ HT • NP • 1				

(Bio)-SiO₂@TiO₂ – Case Studies

XRD



Antibacterial Test

	<i>S. aureus</i>		<i>E. coli</i>	
	MIC ¹ (mg/mL)	MBC ² (mg/mL)	MIC ¹ (mg/mL)	MBC ² (mg/mL)
Bio-SiO ₂ @TiO ₂ • NP • 1	10	>10	10	>10
Bio-SiO ₂ @TiO ₂ • NP • 2	10	>10	>10	>10
SiO ₂ @TiO ₂ • NP • 1	5	>10	5	>10

¹minimum inhibitory concentration (growth)

²minimum bactericidal concentration (bactericidal)

(Bio)-SiO₂@TiO₂ – Case Studies

CS 1.1

- Spray coating at CeNTI: for low amount applied with stand alone unit, later upscale with the pilot plant (not fully yet installed);
- Finalise KDFs for incorporation step

CS 2

- Spray coating at CeNTI (waiting for the membranes to be sent by B4C)
- Sonochemical coating at BIU

CS 6

- SiO₂@TiO₂ was sent to start incorporation test, while the optimization of bio-SiO₂@TiO₂ is ongoing
- RoV observed the presence of dark particulate matter in the material - possibly coming from the ultrasonic probe

New batch produced – 200 g available

(Ref.^a NF • SiO₂@TiO₂ HT • NP • 2)

Set-back with **pressurized reactor (hydrothermal process) and pilot centrifuge**

- TEM, SEM-EDS & antibacterial (BIU), XRD (?) and FTIR (CeNTI)
- Tox and eco-tox evaluation (UNIMB and CNR-IAS: SiO₂@TiO₂ (+ bio-SiO₂))



10L Reator

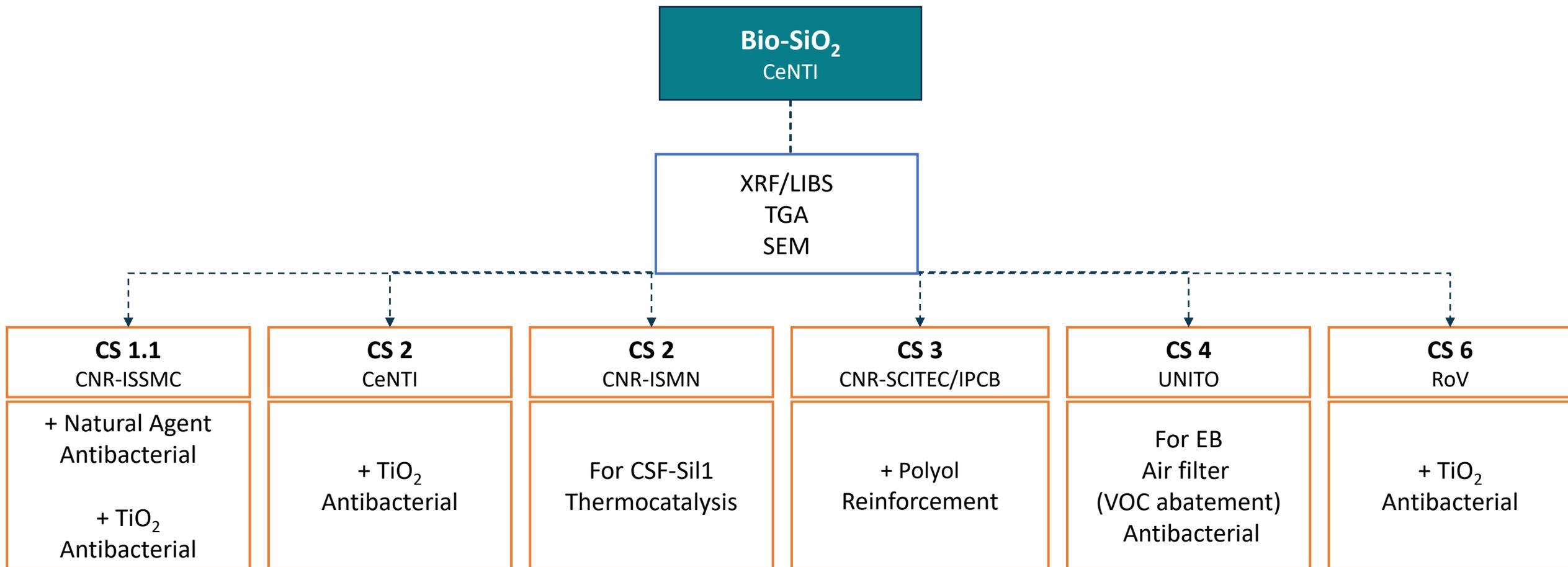


2L Pressurized Reactor

Bio-SiO₂ – Case studies

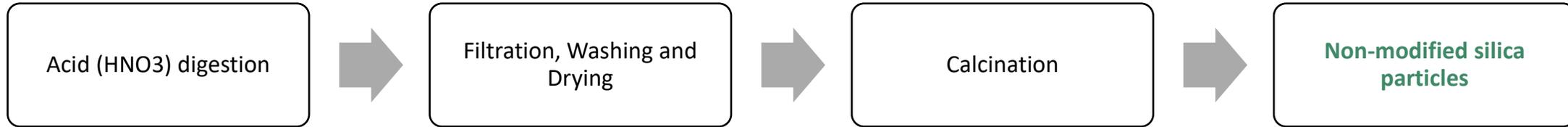
NM
characterization

NEP
characterization

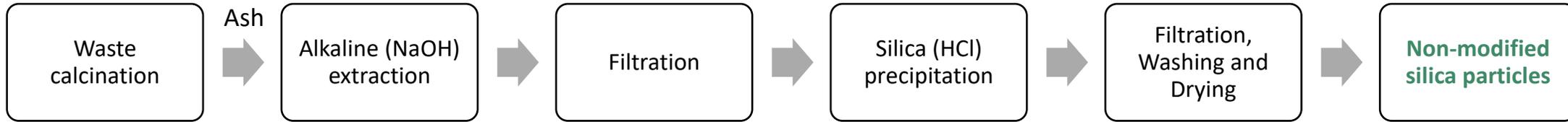


Bio-SiO₂ – Extraction Processes Studied

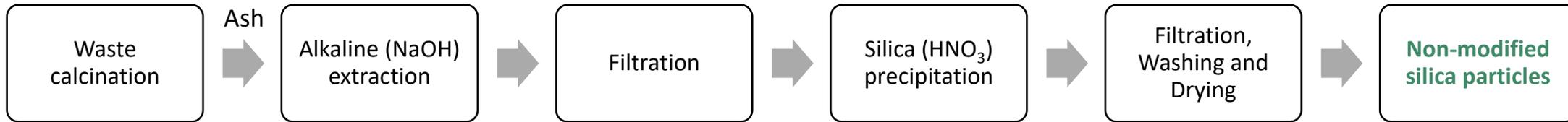
Acid Digestion (AD_HNO₃)



Sol-gel (SG_HCl)

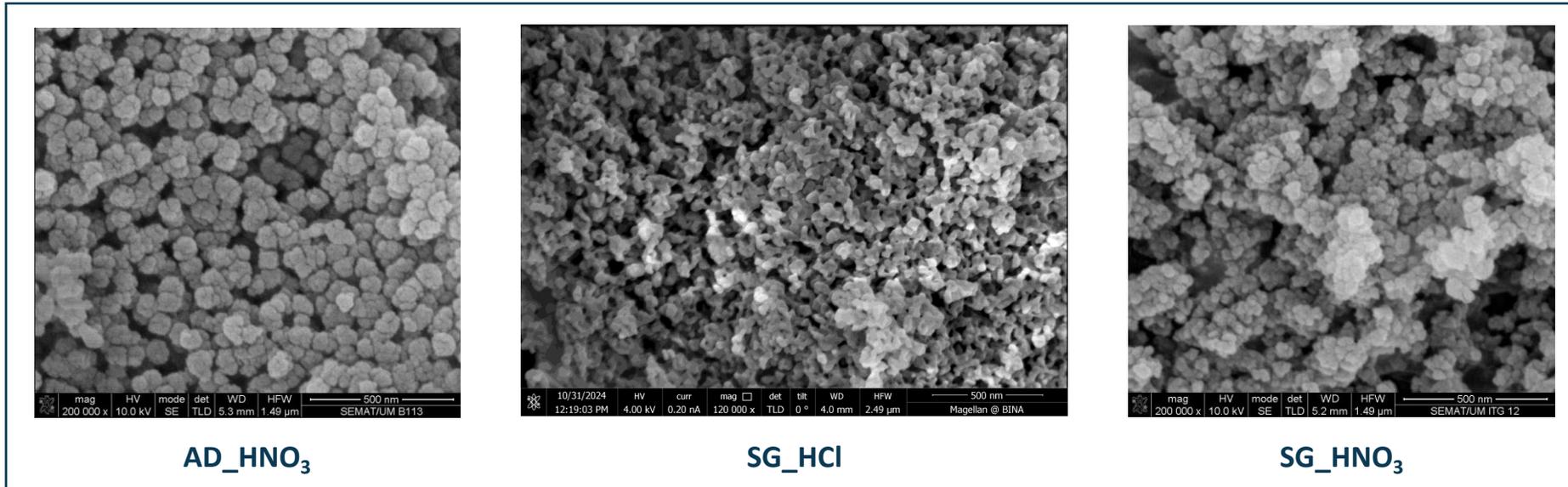


Sol-gel (SG_HNO₃)



Bio-SiO₂ – Characterization

SEM

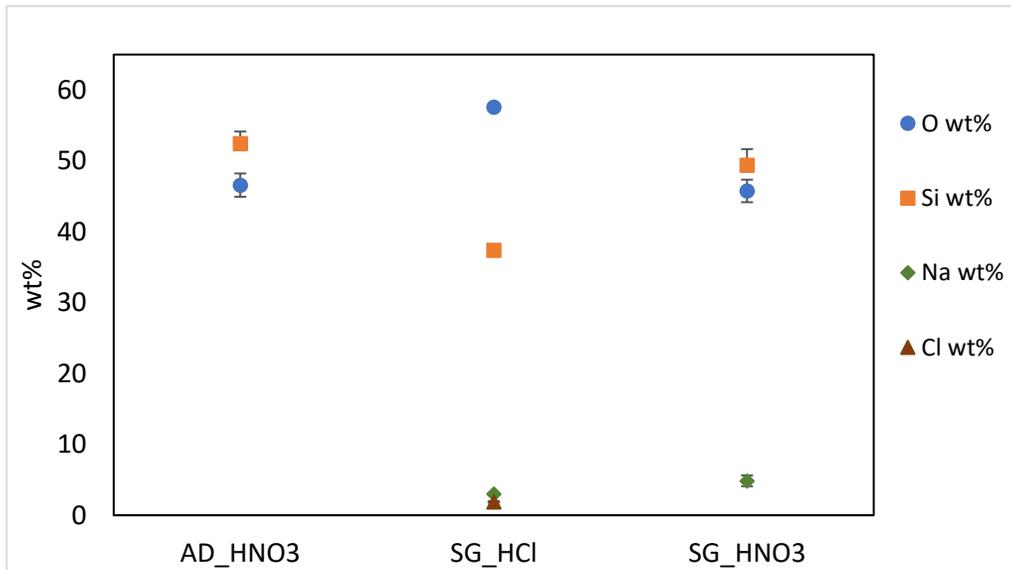


- Acid digestion with HNO₃ results in more well-defined and less aggregated particles, suggesting silica with lower porosity and better growth control;
- The sol-gel method generates more porous structures;
- HNO₃ in the sol-gel process appears to lead to a slightly more ordered structure compared to HCl, with less aggregated particles.

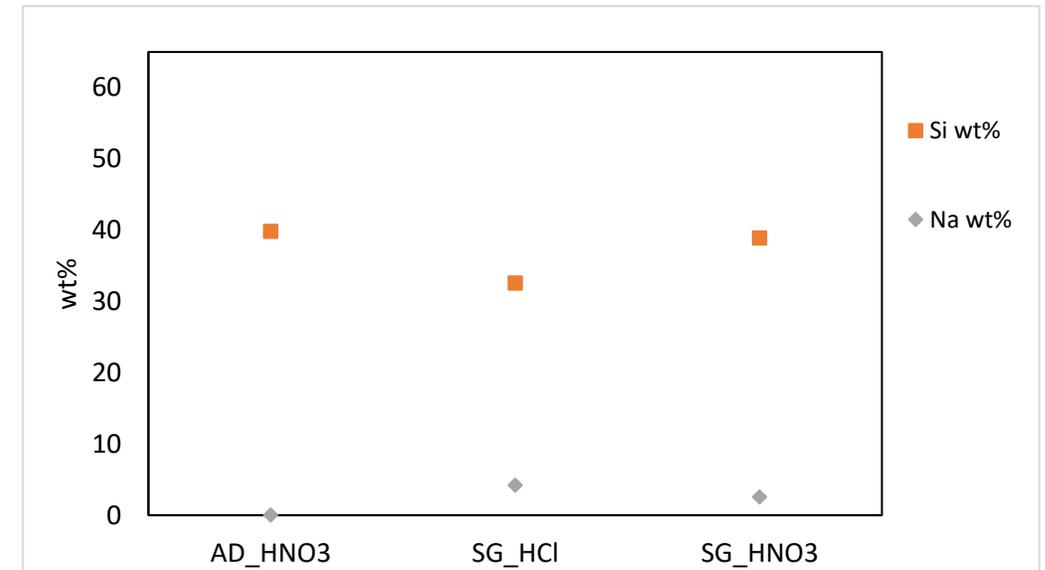
Next step: Evaluate by BET and XRD (partner?)

Bio-SiO₂ – Characterization

EDS



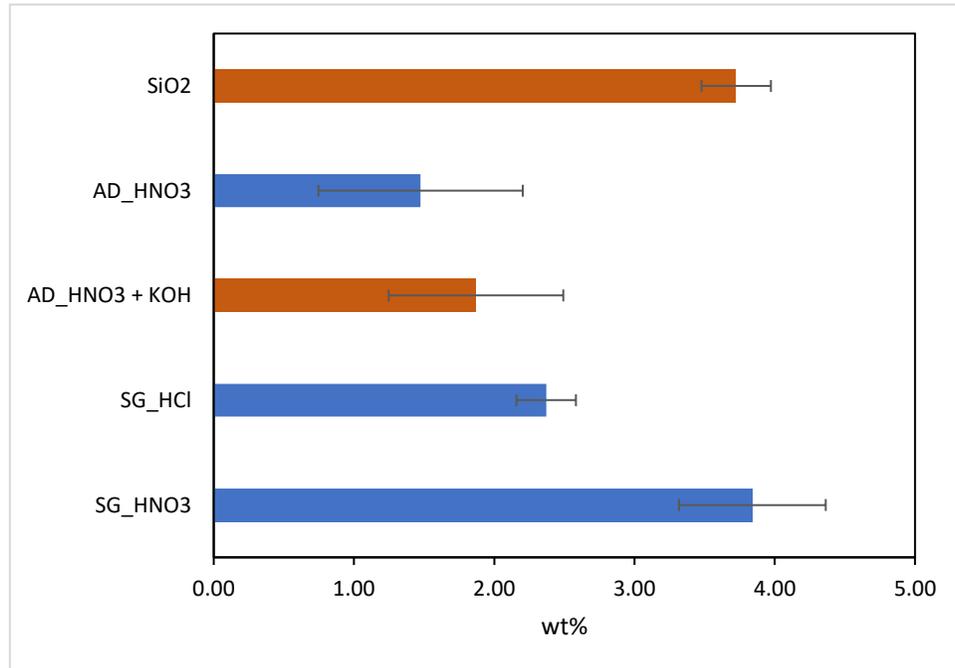
XRF & LIBS



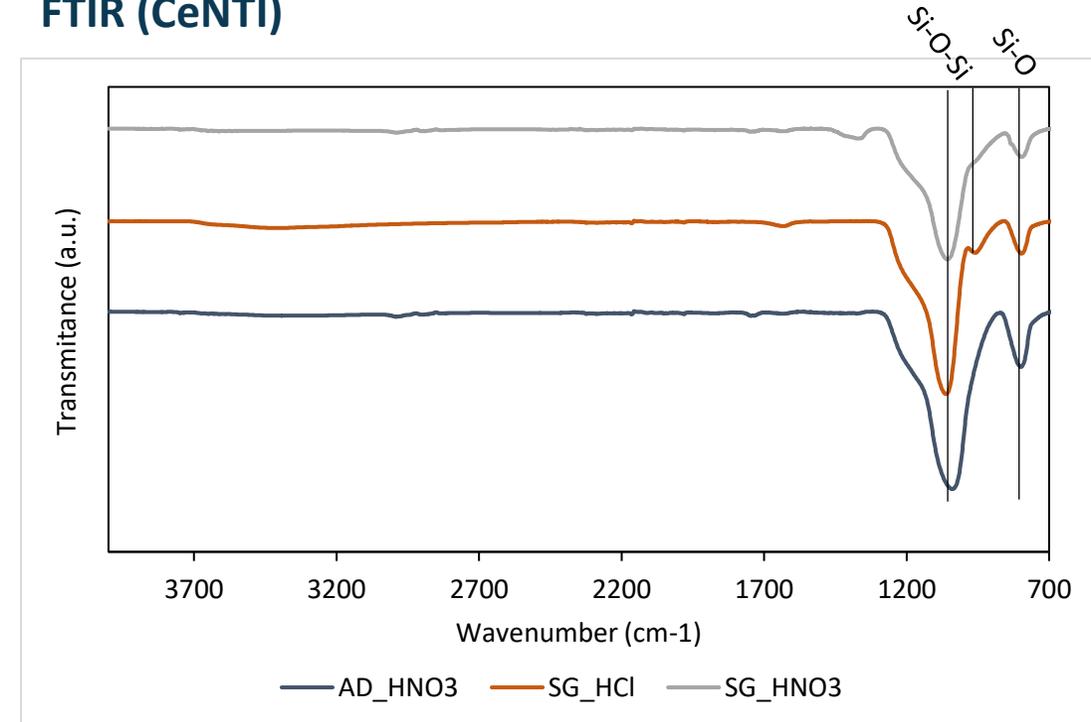
- Acid digestion with HNO₃ (AD_HNO3) produces a purer silica, while the sol-gel method, especially with HCl, tends to generate more porous materials with more impurities.

Bio-SiO₂ – Characterization

-OH groups (mmol/g)



FTIR (CeNTI)

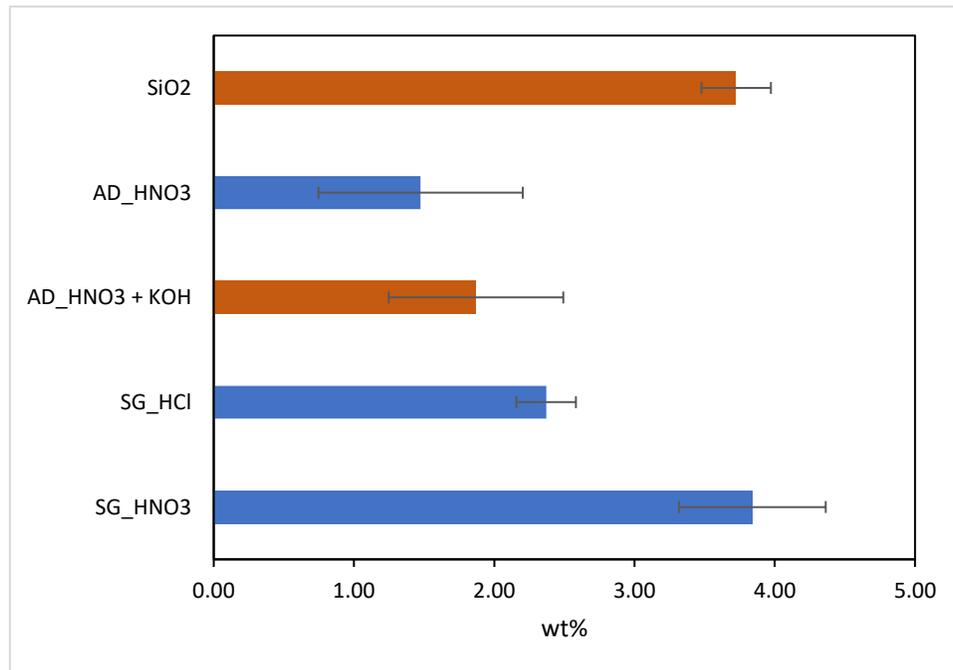


The **sol-gel method**, particularly when **neutralized with different acids**, can significantly influence the silica network structure.

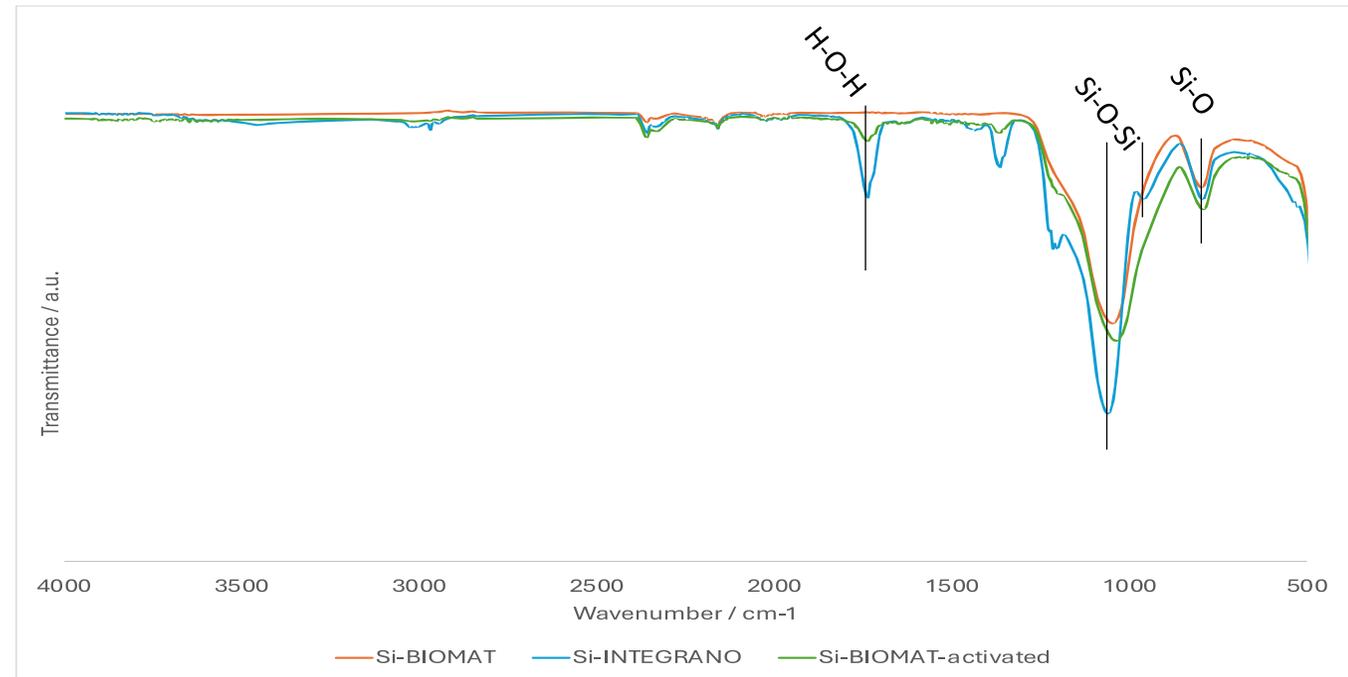
- SG_HCl: Neutralization with HCl may result in more homogeneous particles and a lower density of hydroxyl groups;
- SG_HNO₃: HNO₃ can produce denser networks, reflected by more intense bands at 1100–1000 cm⁻¹ and reduced width at 3600–3200 cm⁻¹.

Bio-SiO₂ – Characterization

-OH groups (mmol/g)



FTIR (CNR-SCITEC)

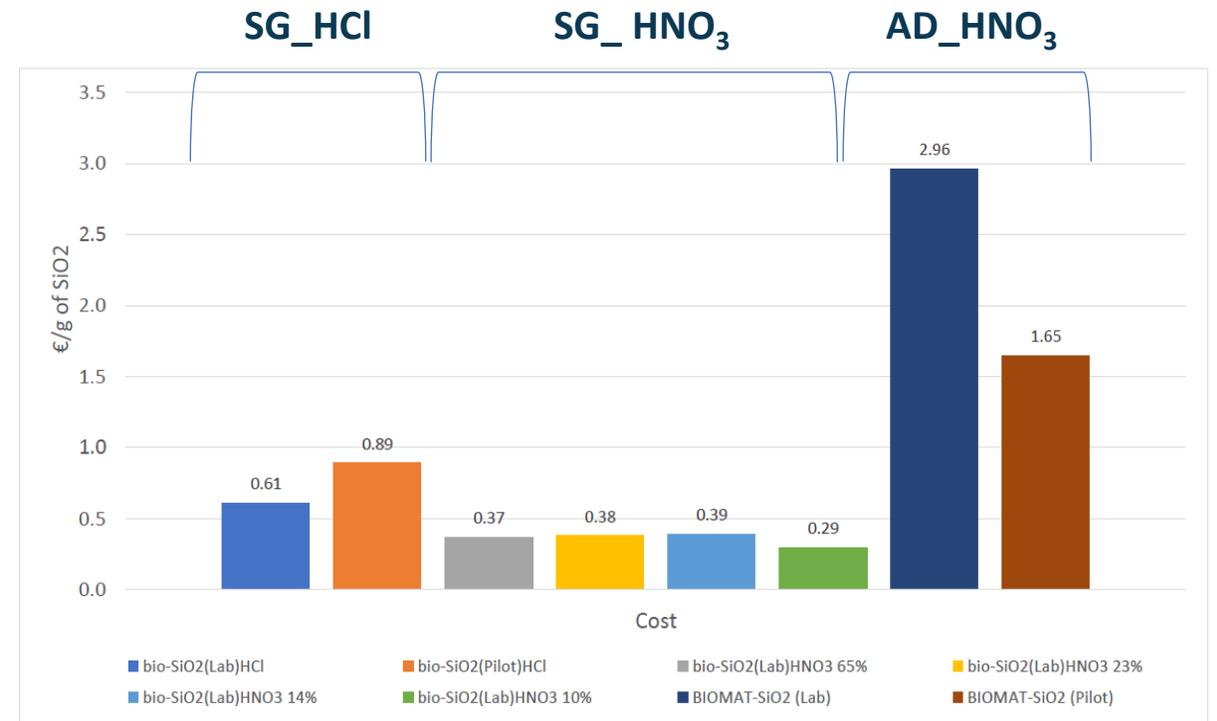
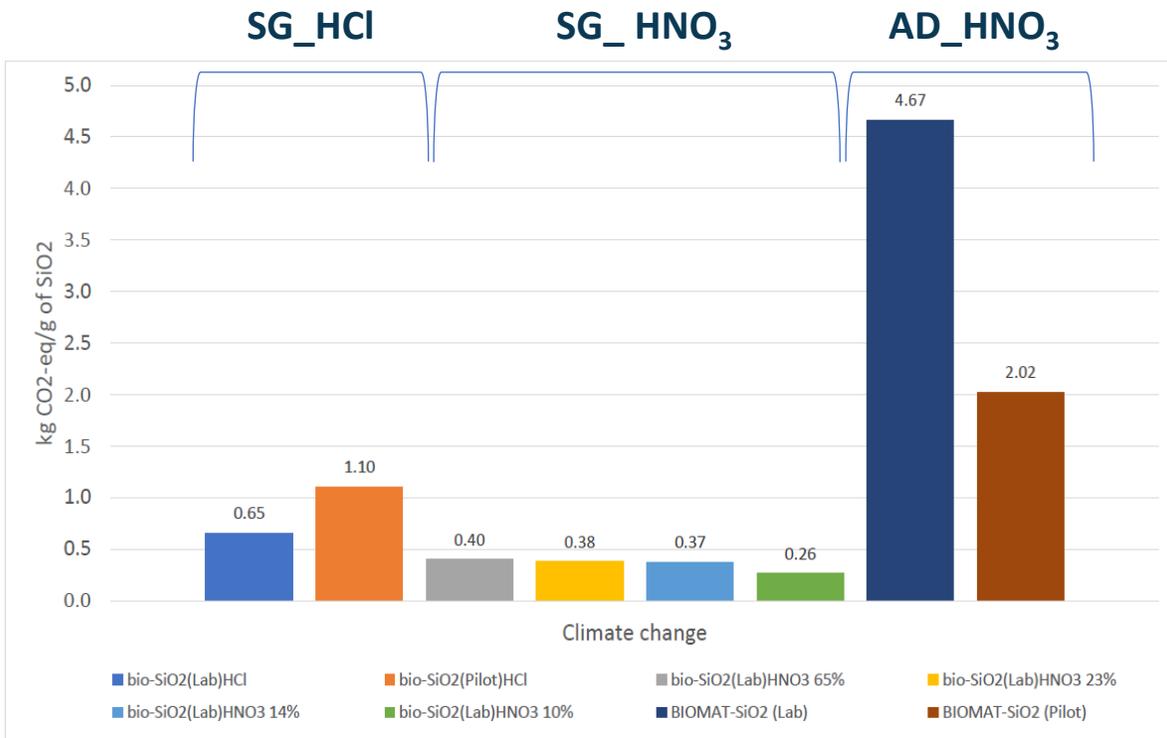


The **sol-gel method**, particularly when **neutralized with different acids**, can significantly influence the silica network structure.

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Bio-SiO₂ – Characterization

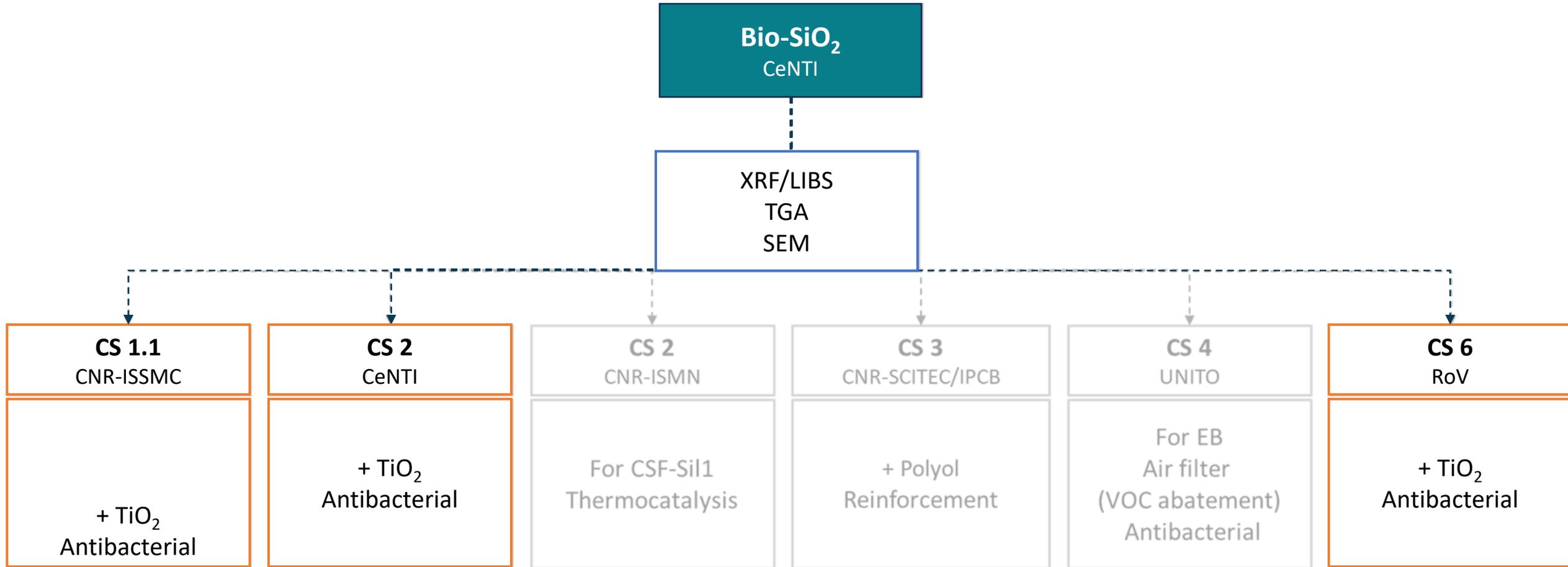
LCA & LCC evaluation



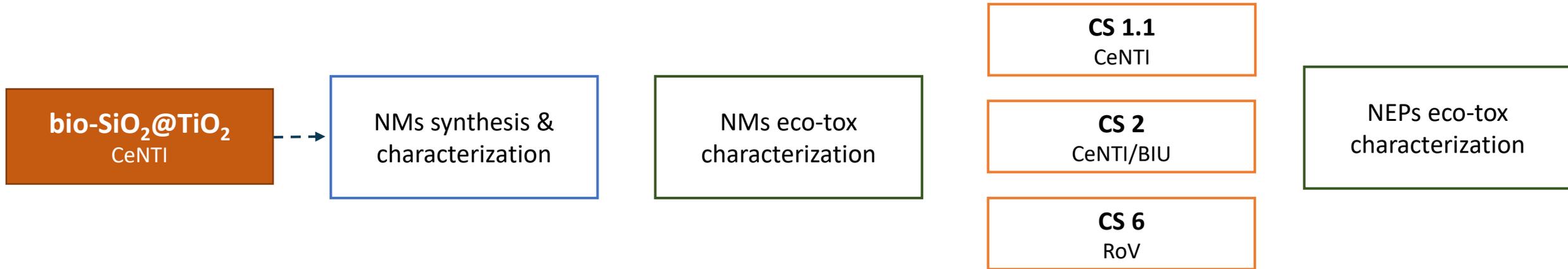
Bio-SiO₂ – Case studies

NM
characterization

NEP
characterization



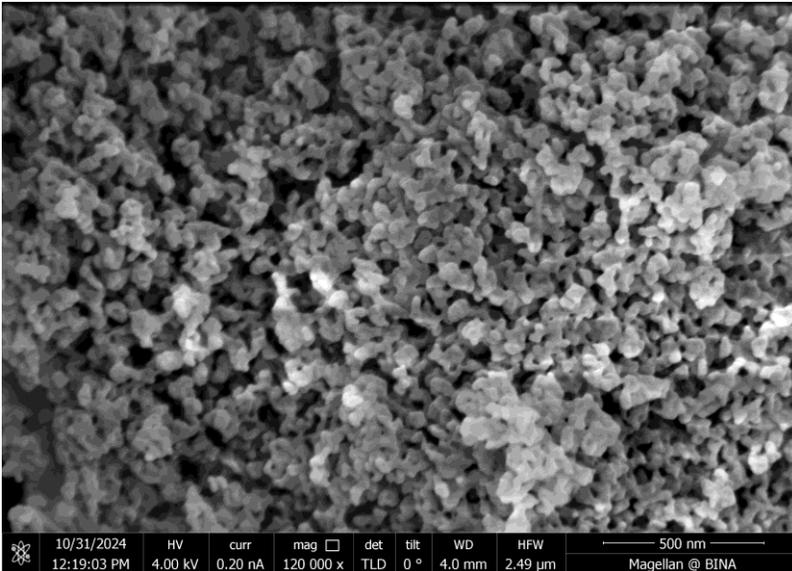
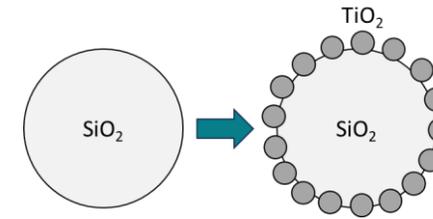
Bio-SiO₂ – Case studies



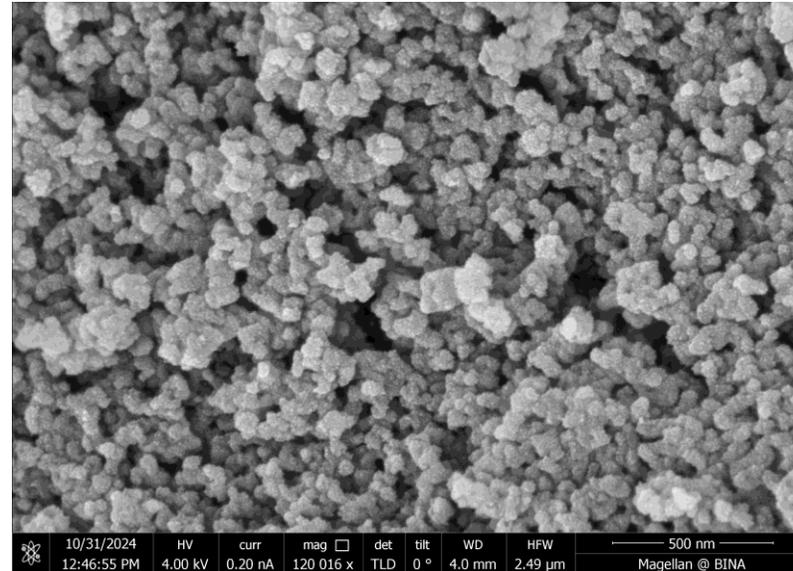
Bio-SiO₂@TiO₂ – Case studies

Optimization 1: Evaluation of bio-SiO₂ dispersion in functionalization with TiO₂

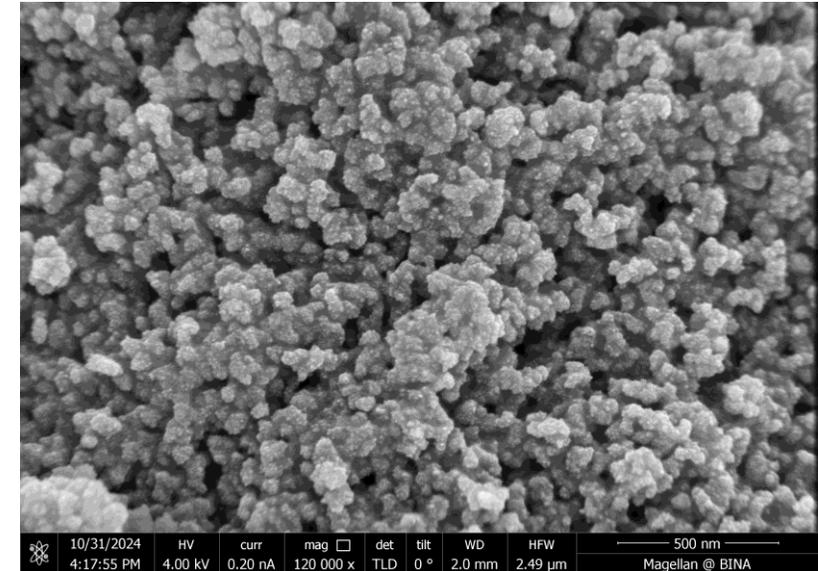
SEM



BioSiO₂



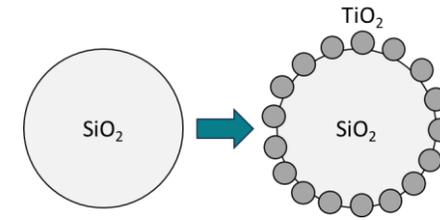
BioSiO₂@TiO₂ – w/out ultrasound



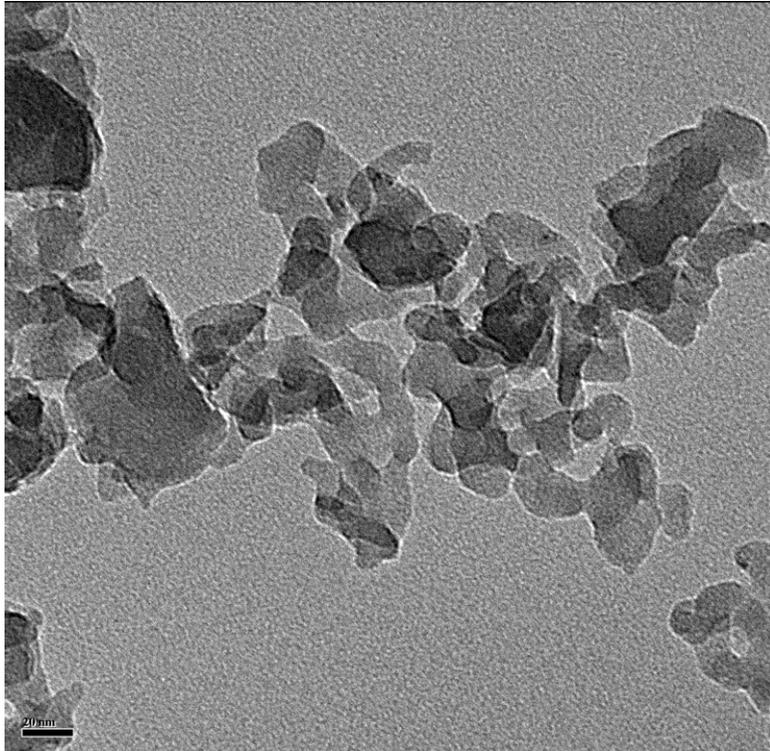
BioSiO₂@TiO₂ – 1h ultrasound

Bio-SiO₂@TiO₂ – Case studies

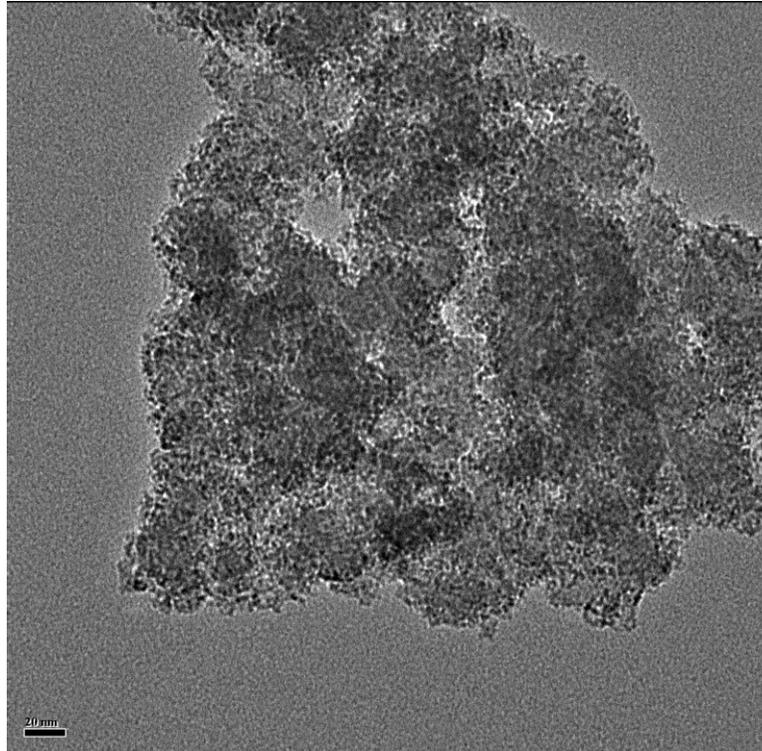
Optimization 1: Evaluation of bio-SiO₂ dispersion in functionalization with TiO₂



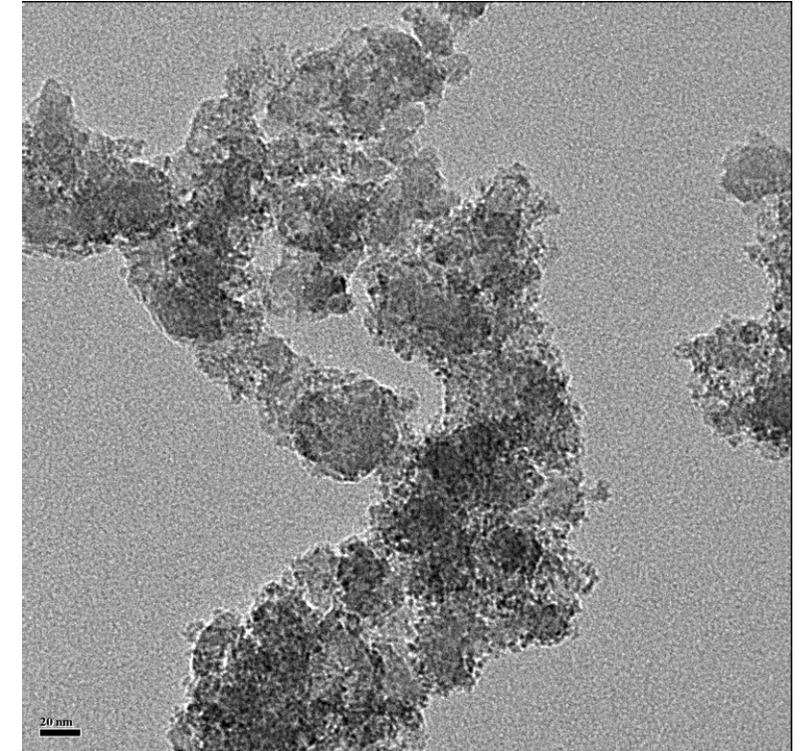
STEM



BioSiO₂



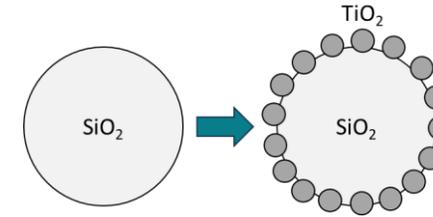
BioSiO₂@TiO₂ – w/out ultrasound



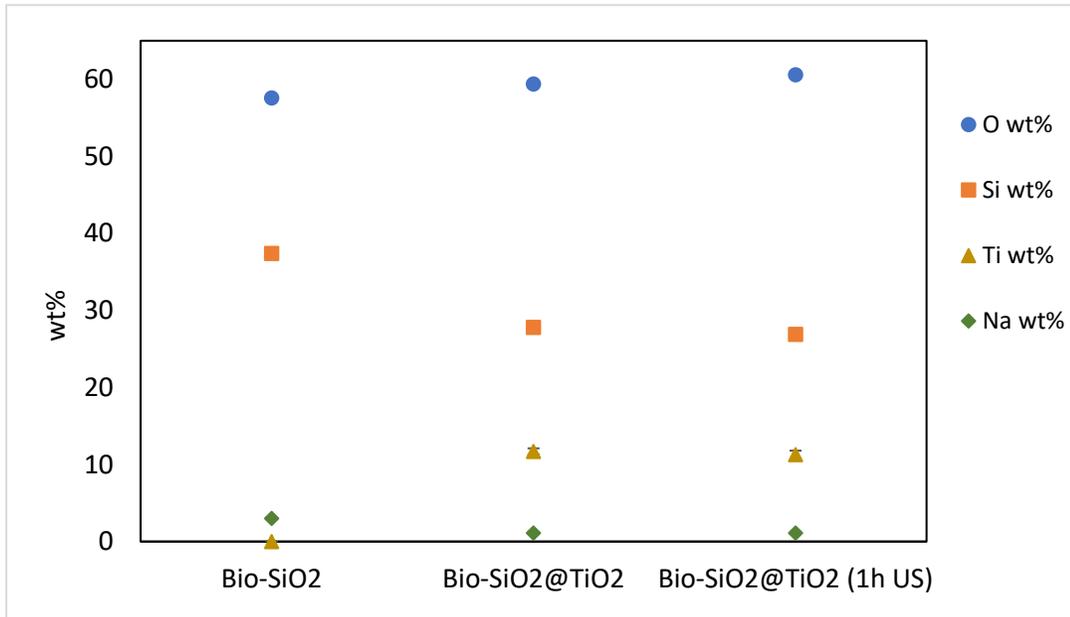
BioSiO₂@TiO₂ – 1h ultrasound

Bio-SiO₂@TiO₂ – Case studies

Optimization 1: Evaluation of bio-SiO₂ dispersion in functionalization with TiO₂



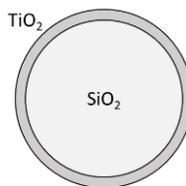
EDS



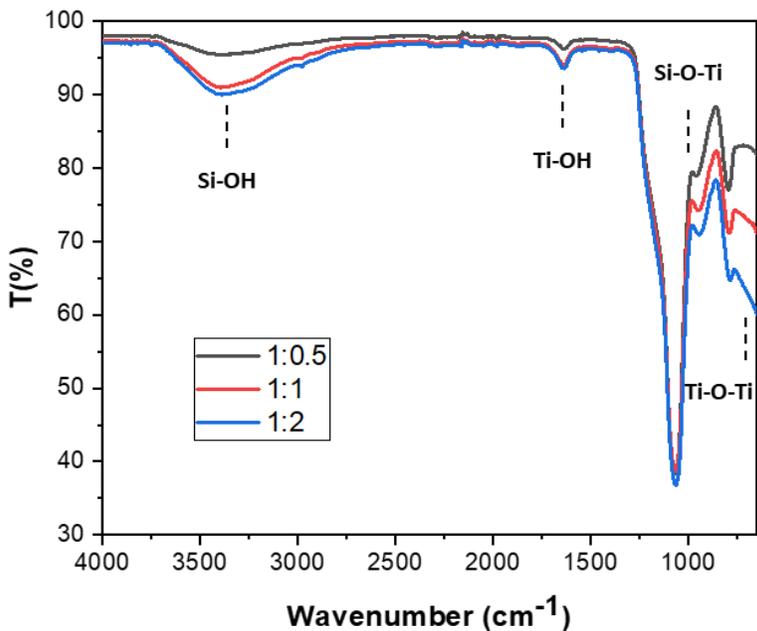
- The Bio-SiO₂ sample exhibits the highest Si concentration, as it consists only biogenic silica;
- After functionalization with TiO₂, the Si concentration decreases, indicating that TiO₂ is partially coating the silica;
- The functionalization of bio-SiO₂ with TiO₂ was confirmed by the presence of titanium in the treated samples;
- The use of ultrasound for 1 hour did not drastically change the elemental composition.

Bio-SiO₂@TiO₂ – Case studies

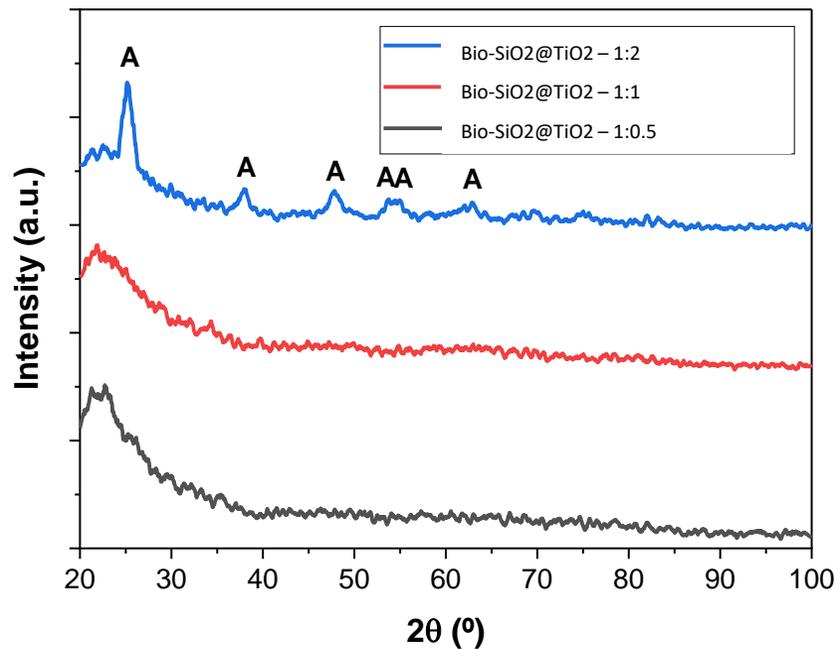
Optimization 2: Evaluation of TiO₂ shell thickness



FTIR-ATR



XRD

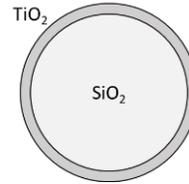


Theoretical TiO ₂ /SiO ₂ ratio	Experimental ¹ TiO ₂ /SiO ₂ ratio
0.5	0.3
1	0.6
2	1.1

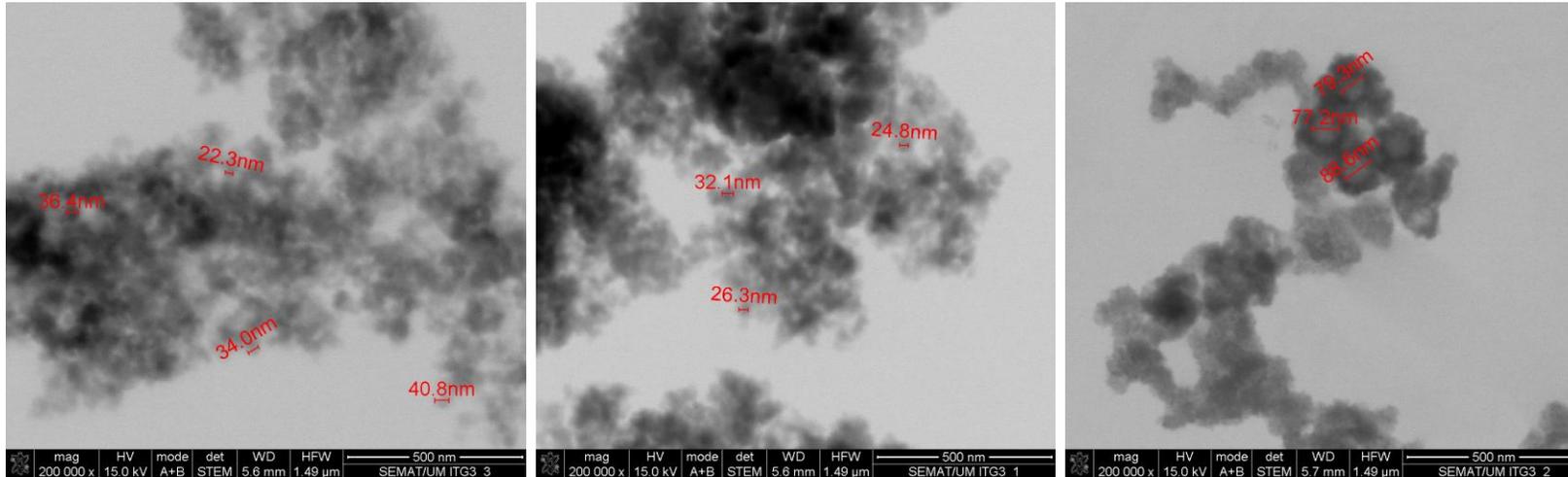
¹Evaluated by XRF

Bio-SiO₂@TiO₂ – Case studies

Optimization 2: Evaluation of TiO₂ shell thickness



STEM



Theoretical TiO ₂ /SiO ₂ ratio	Experimental ¹ TiO ₂ /SiO ₂ ratio	Shell thickness (nm)
0.5	0.3	-
1	0.6	-
2	1.1	39.3 ± 9.8

¹Evaluated by XRF

Bio-SiO₂@TiO₂ – Case studies

Optimization 3: Evaluation of hydrothermal process conditions

DoE matrix

Sample	KDF1 Temperature (°C)	KDF2 Pressure (bar)
P1	40	7
P2	140	7
P3	140	21
P4	40	21
P5	90	14
P6	50	18

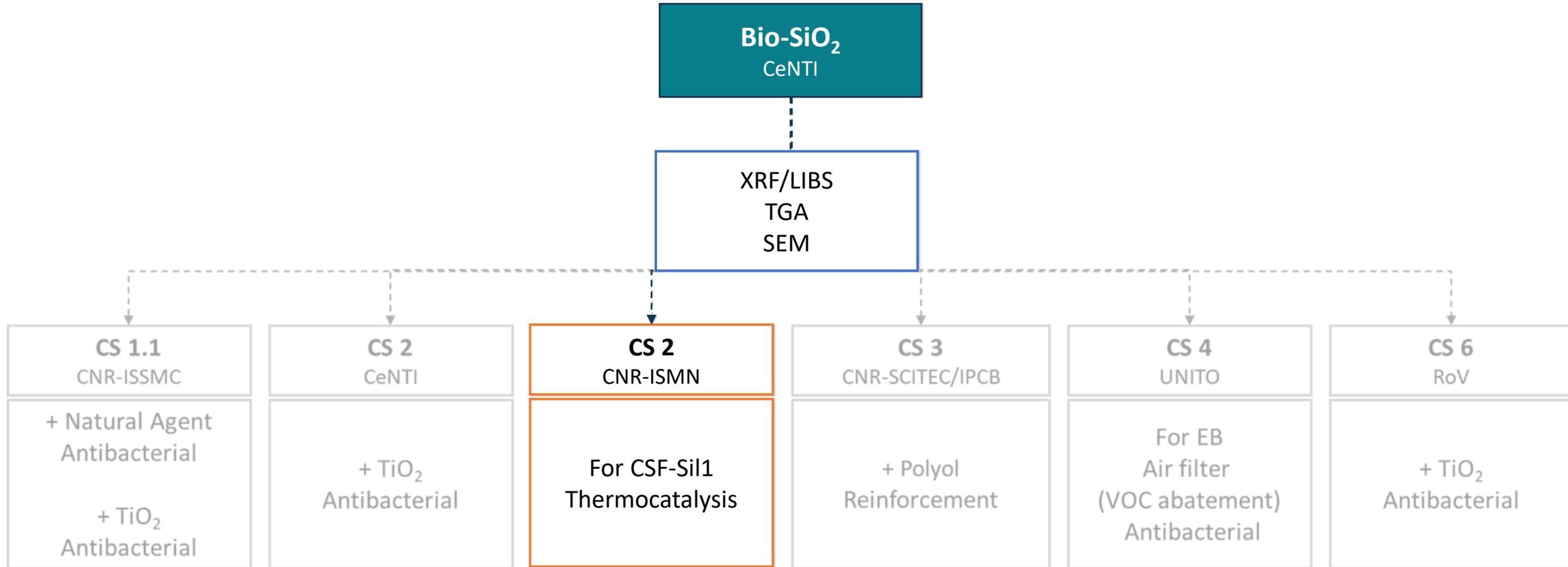
Characterization:

- TEM (size and TiO₂ shell) - BIU
- EDS/XRF (composition) – BIU & CeNTI
- BET (porosity and surface area) –
- XRD (crystallinity) - CNR-ISSMC
- Antibacterial - BIU

Bio-SiO₂ – Case studies

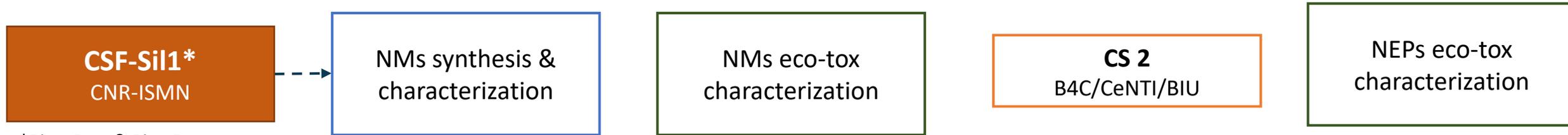
NM
characterization

NEP
characterization



Tasks 2.1 – 2.3

Overview of the NMs and NEPs targeted



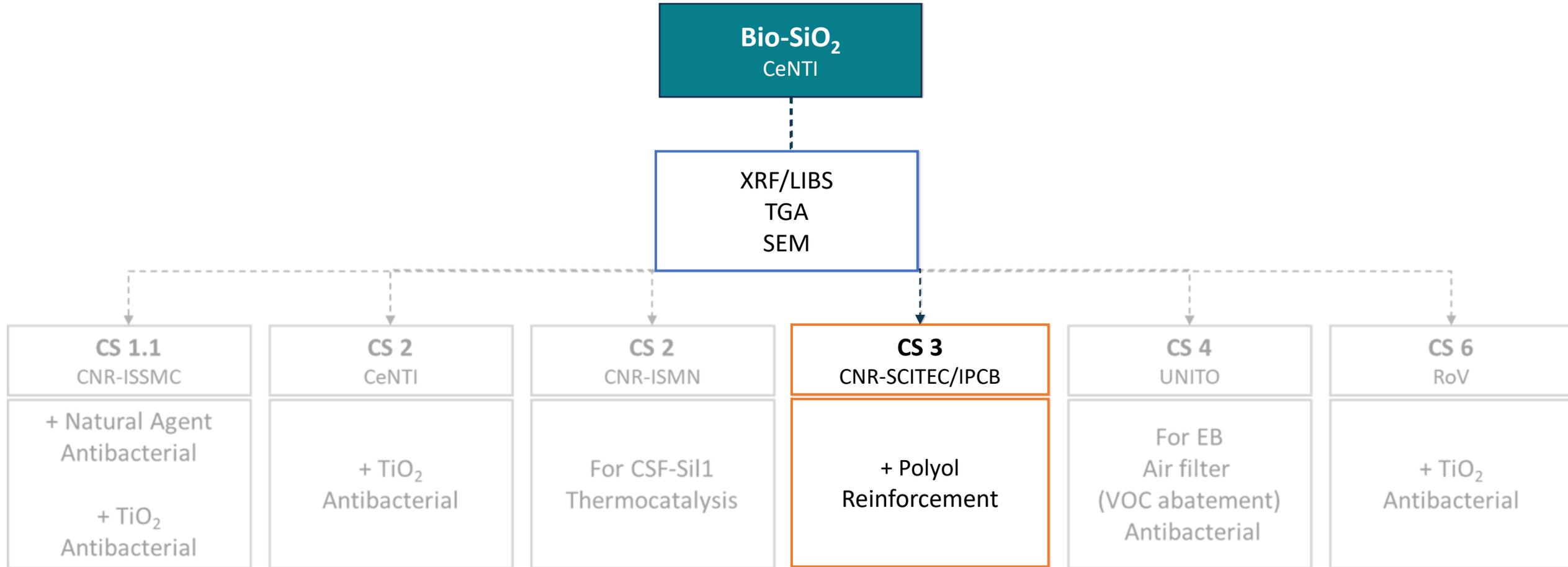
*Rice, Rust & Rice-Rust

- Bio-SiO₂-rice husk, Fe-rust waste or both of them were investigated as waste precursors replacing synthetic (organosilica precursors) or iron nitrate commercial reagent – with pH (5) and reducers-to-oxidizers ratio (1.6) fixed.
- Only sample with bio-SiO₂ + Fe-nitrate presented antibacterial activity
- Seven samples (six + replica) of the optimum sample (bio-SiO₂ + Fe-nitrate) was prepared following a DoE matrix with **two KDFs**:
- KDF1: pH
- KDF2: reducers-to-oxidizers ratio

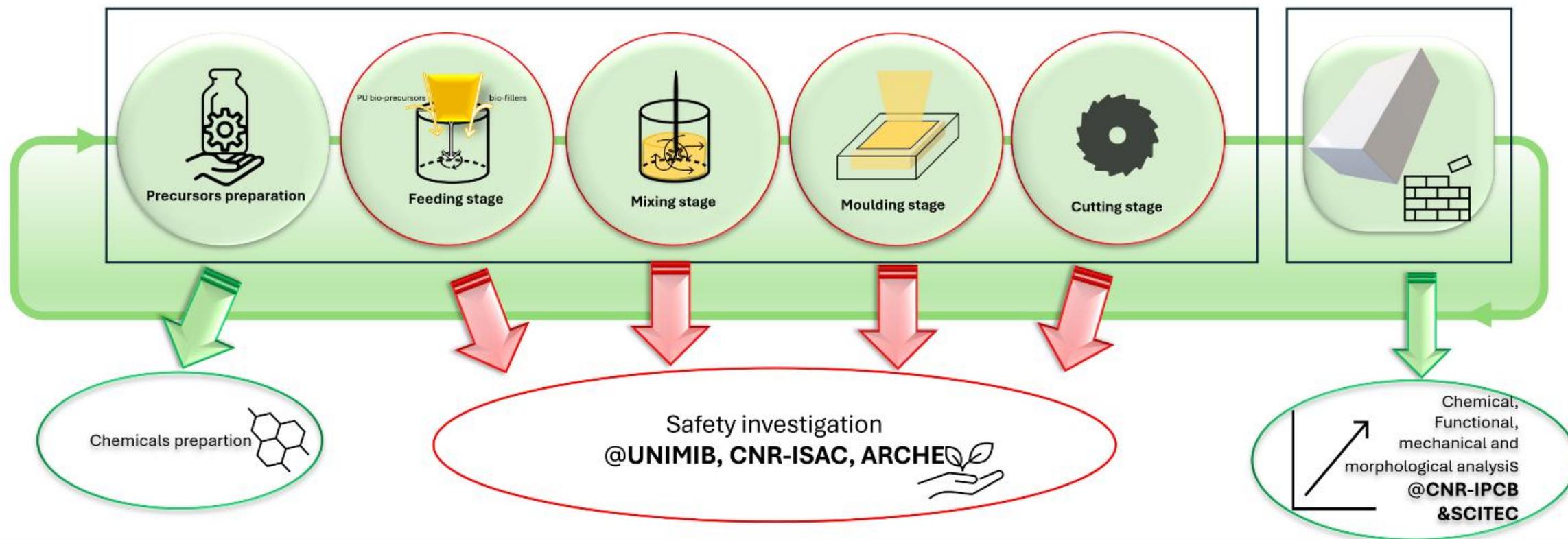
Bio-SiO₂ – Use in case studies

NM
characterization

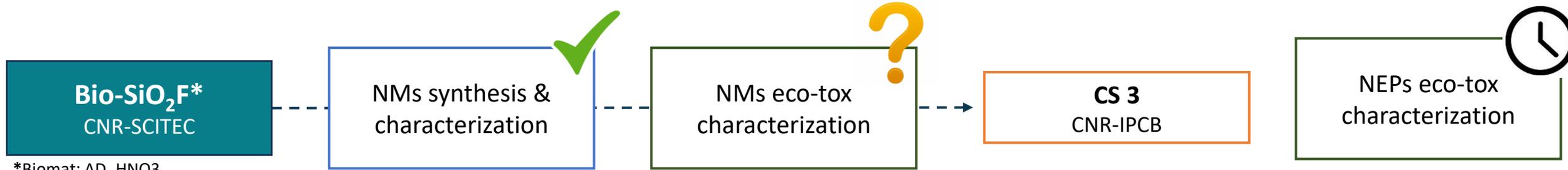
NEP
characterization



Bio-SiO₂ – CS3: Large-scale production of composite PU foam



Bio-SiO₂ – CS3: Large-scale production of composite PU foam



*Biomat: AD_HNO3

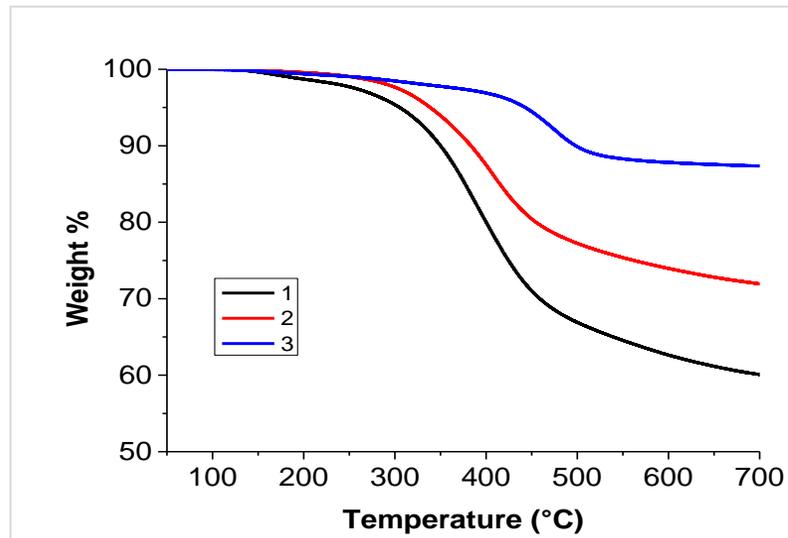
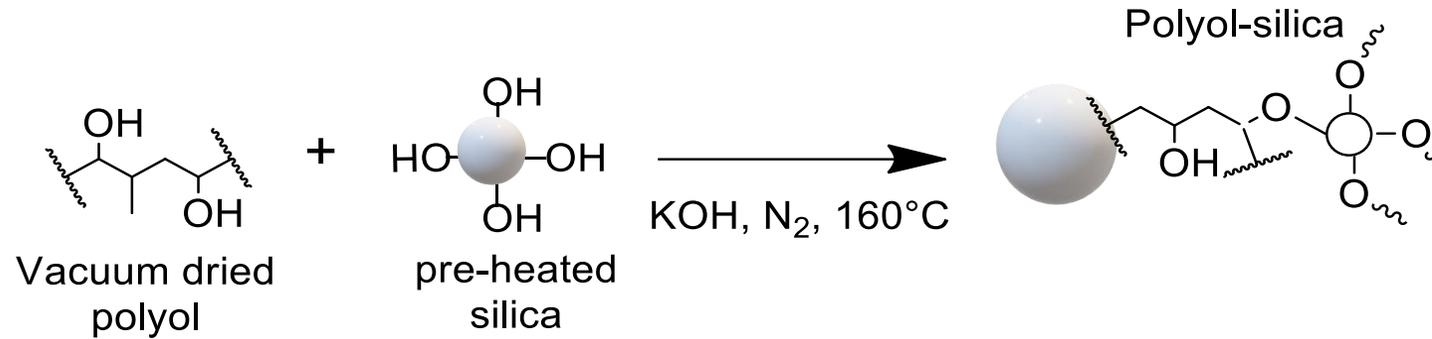
- Evaluation of the effect of **design (1)** and **re-design (3)** on final properties of nanocomposite PU foams and safety production:

Open mould to **mould with lid** configuration
 Bio-SiO₂@F/**Diatomite** to Bio-SiO₂@F/**Gas beton**

- Bio-SiO₂@F/Diatomite and open mould** (ref^a material)
- Bio-SiO₂@F/Gas beton and open mould
- Bio-SiO₂@F/Diatomite and mould with lid
- Bio-SiO₂@F/Gas beton and mould with lid

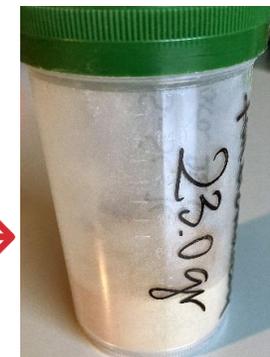


Bio-SiO₂ – CS3: Large-scale production of composite PU foam

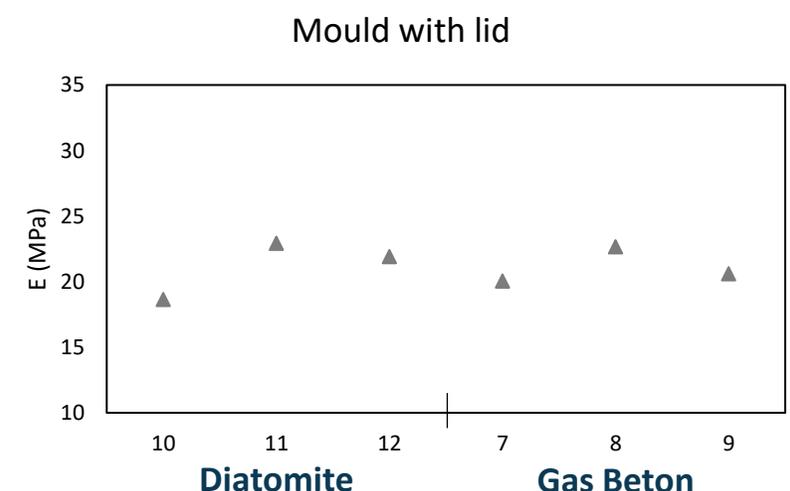
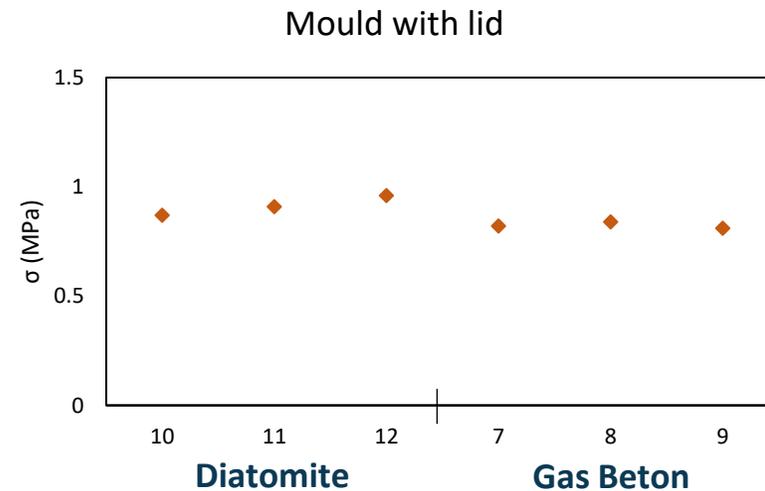
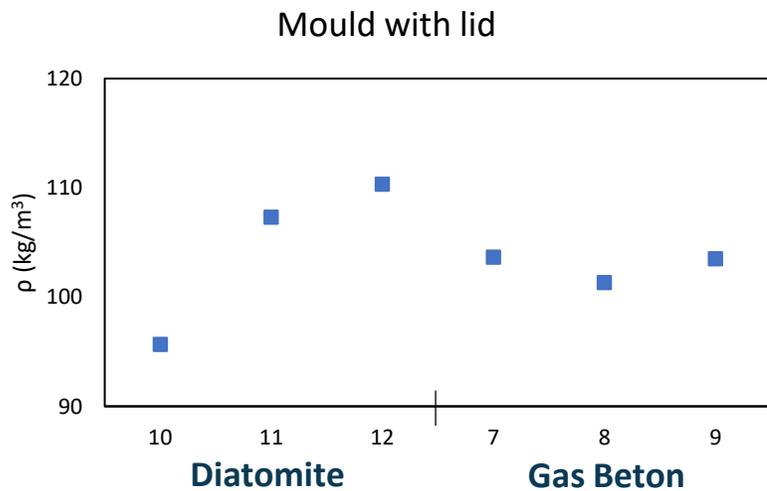
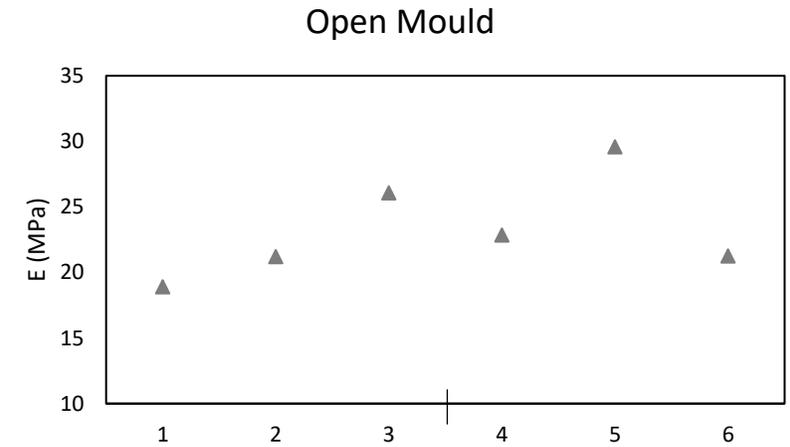
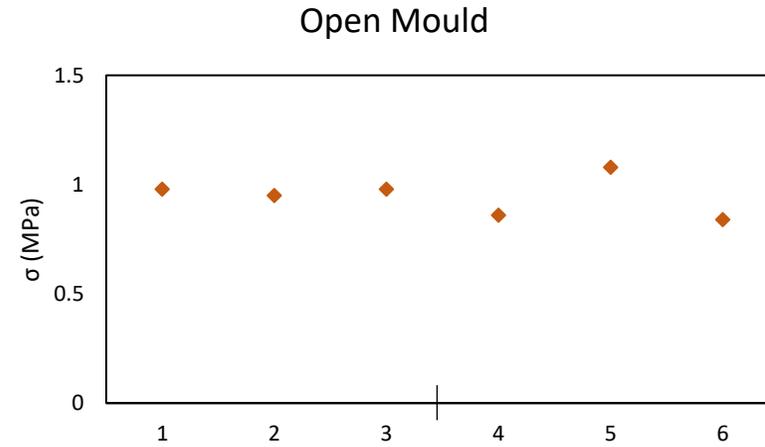
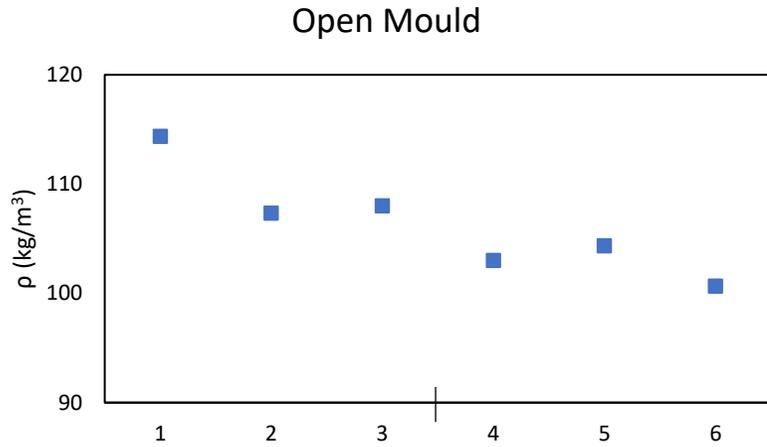


Sample (Bio-SiO ₂ F)	Amount of grafted polyol, wt%
[1]	64
[2]	42
[3]	10

Selected



Bio-SiO₂ – CS3: Large-scale production of composite PU foam



Bio-SiO₂ – CS3: Large-scale production of Composite PU foam

Conclusions:

Effect of mould type:

- The **mould with lid** provides **greater consistency in density and mechanical properties**, indicating better control over the foam formation process.

Impact of reinforcement (Diatomite vs. Gas Beton):

- Both reinforcements exhibit similar properties, but **gas beton appears to offer higher density and better mechanical properties** in some cases.

Property uniformity:

- Using a **mould with lid is more suitable for achieving homogeneous results** in terms of density and mechanical strength.
- Small differences in values suggest that **the reinforcing materials (diatomite and gas beton) have minimal influence** on the overall properties under the tested conditions.

Thermal conductivity:

- So far, the **thermal conductivities are not different** according to the N-filler and mould configuration (**0.034 W/mk**).

Task 2.4

Characterisation and Detection of NMs and NEPs in real-case LC scenarios

- Task 2.4.1 – NMs characterisation in their native form and after environmental interaction.
- Task 2.4.2 – Detection of NMs emitted into the environment (synthesis & incorporation).
- Task 2.4.3 – Experimental simulation of NMs Emission in environmental compartments with complex matrices (use & EoL).

Task 2.4.2

Dedicated field campaigns will be set up to obtain the NMs emissions into the environment by sampling process:

- Airborne NMs detection to monitor emission and occupational exposure.
- Direct-reading instruments: Scanning Mobility Particle Sizer (SMPS); Optical Particle Counter (OPC); Condensation particle Counter (CPC).
- Offline analysis: gravimetric analysis, aerosol collection on filters for SEM observations.
- Simultaneous measurements at near field (NRF) and far field (FRF) positions.

Bio-SiO₂ – CS3: Large-scale production of Composite PU foam

Task 2.4.2 – Field campaign

Pilot Line: Low Density Casting Machine of CNR-IPCB

Processes Analyzed (in Triplicate):

- Bio-SiO₂@F/Diatomite & Mould without Lid Configuration (x3)
- Bio-SiO₂@F/Gas Beton & Mould without Lid Configuration (x3)
- Bio-SiO₂@F/Gas Beton & Mould with Lid Configuration (x3)
- Bio-SiO₂@F/Diatomite & Mould with Lid Configuration (x3)

Total Processes Measured: 12, plus the background measurements taken during lunch breaks and one night.

Measurement Phases:

- Weighing Phase
- Loading Phase
- Casting Phase
- Cutting Phase

Focus: Identify whether a process is associated with lower emissions.



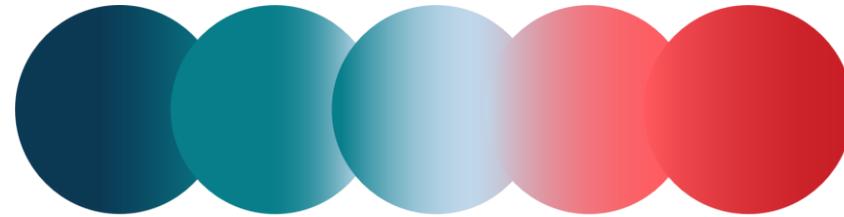
To be discussed

1. Antibacterial evaluation

- Which partners do they perform antibacterial tests, and which methods are available?
- What methods and strains do we need for each case study/application?



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INTEGRANO

**MULTIDIMENSIONAL INTEGRATED QUANTITATIVE APPROACH TO ASSESS SAFETY AND
SUSTAINABILITY OF NANOMATERIALS IN REAL CASE LIFE CYCLE SCENARIOS USING
NANOSPECIFIC IMPACT CATEGORIES**

WP2

Experimental Data Generation: NMs provision and characterisation M-Measure (I)

12M Annual General Meeting

Turin - Italy
29-30 January 2025