Electronic Supplementary Information

Transforming Waste Fish Bones to Nanoparticles with Ultrasound and Aqueous Organic Acids

Sarah Boudreau,[a] Sabahudin Hrapovic,[b] Emma McIsaac,[a] Edmond Lam,[b] Fabrice Berrué,[c]* and Francesca M. Kerton[a]*

a. Department of Chemistry, Memorial University, St. John's, NL, Canada; fkerton@mun.ca
b. Aquatic and Crop Resource Development, National Research Council Canada, Montreal, QC, Canada.
c. Aquatic and Crop Resource Development, National Research Council Canada, Halifax, NS, Canada; fabrice.berrue@nrc-cnrc.gc.ca

fkerton@mun.ca

Table S1. Size, PDI,[a] and zeta-potential of nHAP particles created using different milling times and sonication conditions.

Entry	Mill time (min)	Sonicated?	Size (nm)	SD[a]	PDI[a]	SD[a]	Zeta (mV)	SD[a]
S1	60	No[b]	1,509	141.2	0.823	0.117	-3.12	0.239
S2	120	No[b]	1,658	244.7	0.802	0.184	-4.62	0.134
S3	180	No[b]	2,666	201	0.858	0.078	-1.02	0.145
S4	240	No[b]	1,882	139.5	0.966	0.031	-6.88	0.323
S5	60	Yes[c]	1,391	358.8	0.843	0.138	-10.8	0.551
S6	180	Yes[c]	1,288	66.7	0.666	0.089	-9.56	0.476
S7	240	Yes[c]	1,151	41.4	0.764	0.073	-13.5	1.01

a. Abbreviations – standard deviation, SD; polydispersity index, PDI.
b. Unsonicated samples were prepared by dispersing 10 mg sHAP in 10 mL water by vortexing for 1 min.
c. 10 mg sHAP sonicated in 10 mL water for 15 min and centrifuged for 15 min at 6,000 rpm with the supernatant decanted for analysis and the pellet discarded.

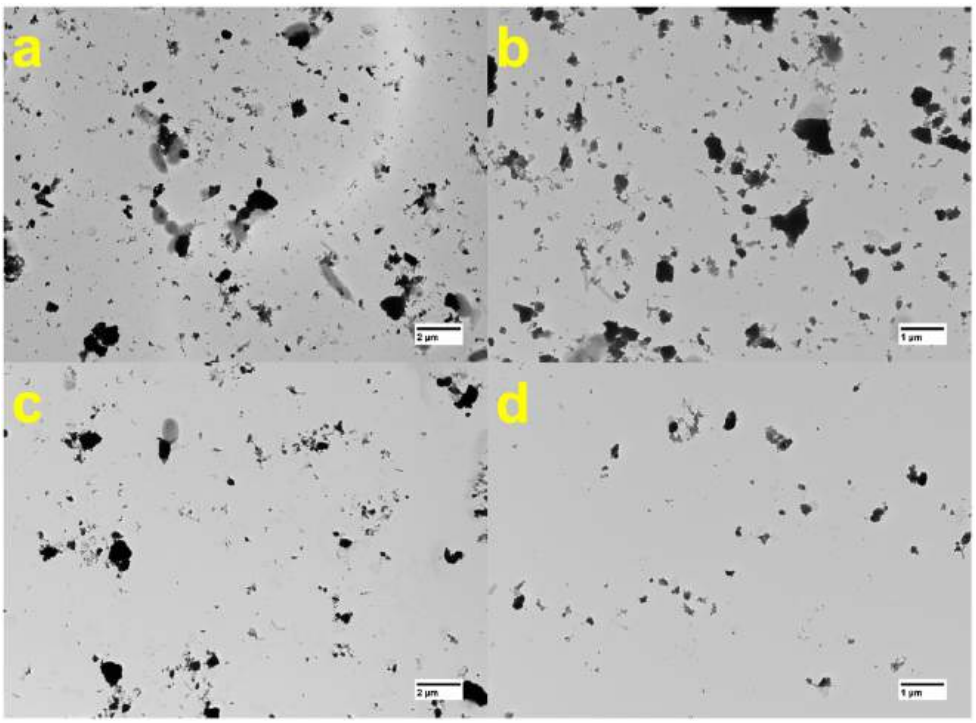

Figure S1. TEM images of sHAP pulverized in a ball mill for 1 (a, entry S1, scale bar: 2 μm), 2 (b, entry S2, scale bar: 1 μm), 3 (c, entry S3, scale bar: 2 μm), and 4 h (d, entry S4, scale bar: 1 μm).

Table S2. Size, PDI,[a] and zeta-potential of nHAP particles created by sonicating 10 mg sHAP in 10 mL water for 15 min and centrifuging for 15 min at 6,000 rpm. The supernatant was decanted for analysis and the pellet discarded.

Entry	Centrifuge time (min)	Size (nm)	SD[a]	PDI[a]	SD[a]	Zeta (mV)	SD[a]
S8	5	840.1	157	0.655	0.088	-0.441	0.187
S9	15	659.7	125.6	0.452	0.060	-8.14	0.318
S10	30	1,554	637.3	0.913	0.125	-9.14	0.422
S11	45	1,492	304.8	0.811	0.126	-12.1	1.76
S12	60	699.6	62.02	0.536	0.056	-9.45	0.454

a. Abbreviations – standard deviation, SD; polydispersity index, PDI.

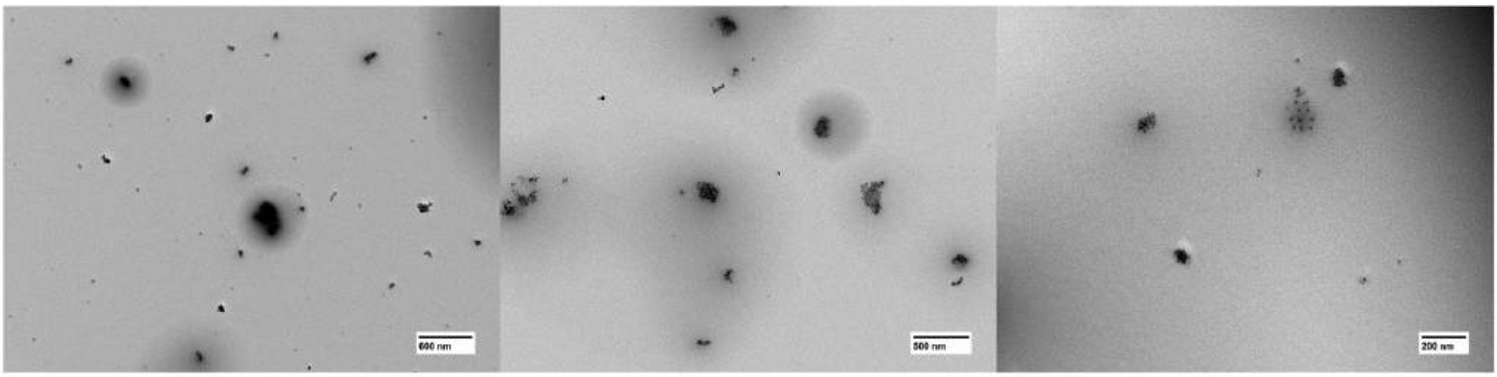

Figure S2. TEM images of nHAP particles synthesized by sonicating 10 mg sHAP in 10 mL 5% oleic acid for 15 min (entry 1; left, scale bar: 600 nm; middle, scale bar: 500 nm; right, scale bar: 200 nm).

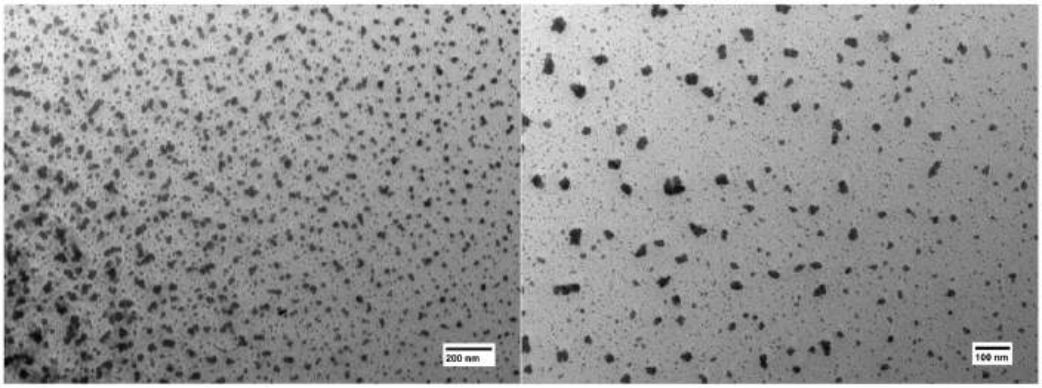

Figure S3. Additional TEM images of nHAP prepared by ultrasound in water used for size distribution histograms (left, scale bar: 200 nm; right, scale bar: 100 nm).

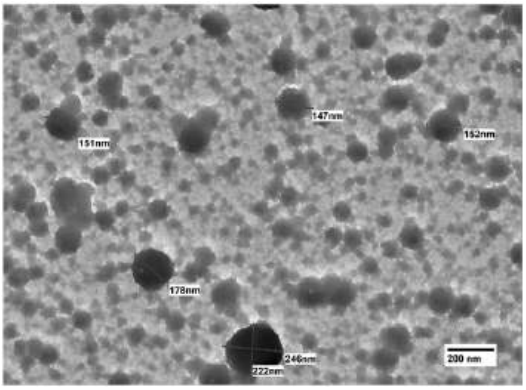

Figure S4. Additional TEM image of nHAP prepared by ultrasound in 5% acetic acid used for size distribution histograms (scale bar: 200 nm).

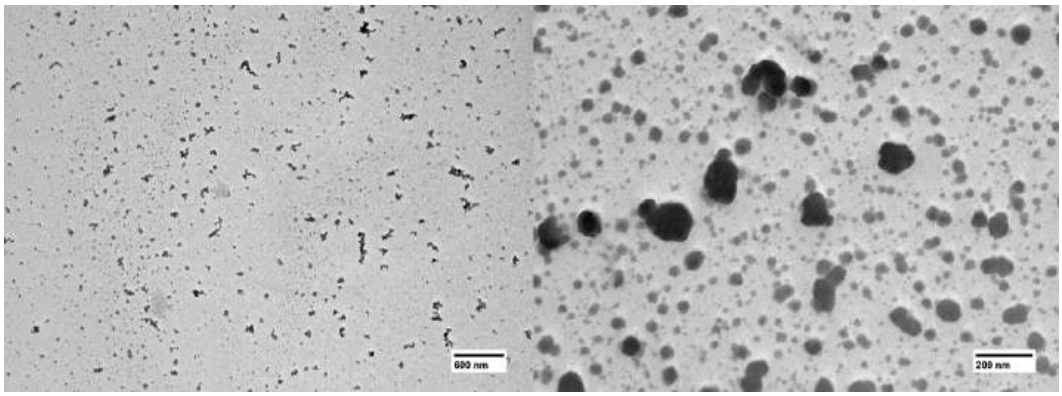

Figure S5. Additional TEM images of nHAP prepared by ultrasound in 5% propanoic acid used for size distribution histograms (left, scale bar: 600 nm; right, scale bar: 200 nm).

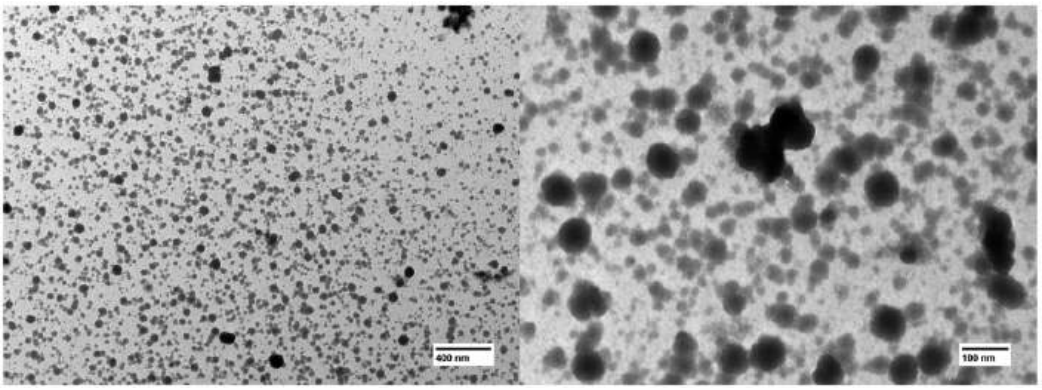

Figure S6. Additional TEM images of nHAP prepared by ultrasound in 10% propanoic acid for 15 min used for size distribution histograms (left, scale bar: 400 nm; right, scale bar: 100 nm).

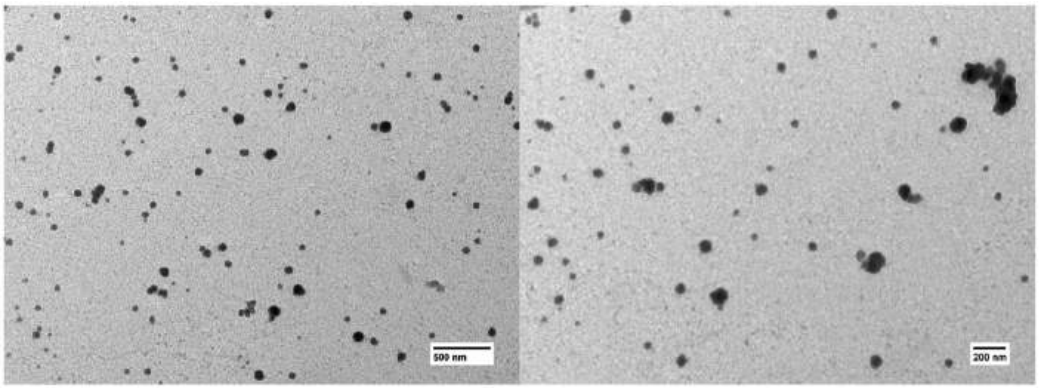

Figure S7. Additional TEM images of nHAP prepared by heating at 200 °C for 24 h and ultrasound in 10% propanoic acid for 15 min used for size distribution histograms (left, scale bar: 500 nm; right, scale bar: 200 nm).

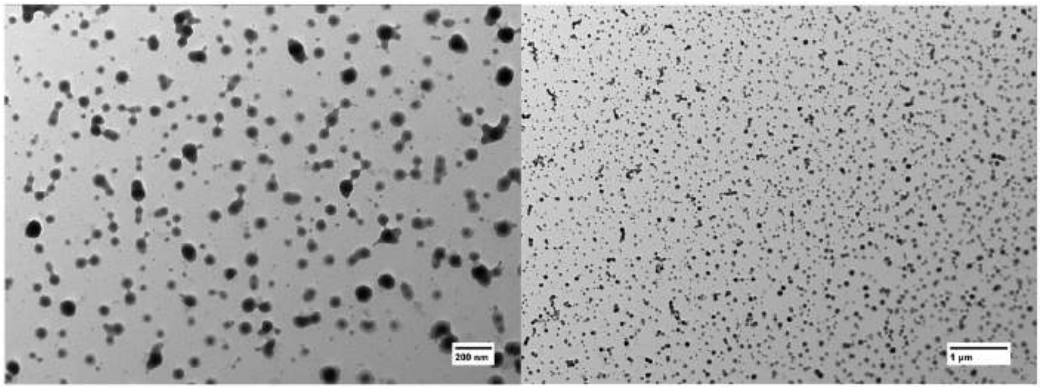

Figure S8. Additional TEM images of nHAP prepared by ultrasound in 10% propanoic acid for 60 min used for size distribution histograms (left, scale bar: 200 nm; right, scale bar: 1 µm).

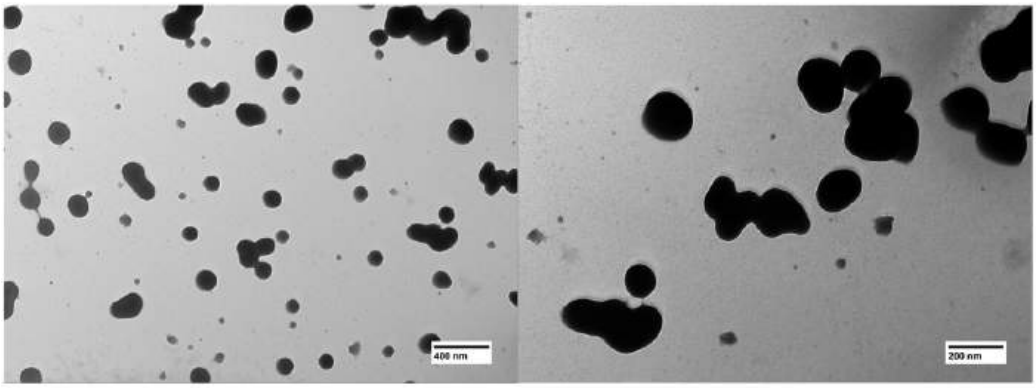

Figure S9. Additional TEM images of nHAP prepared by heating at 200 °C for 24 h and ultrasound in 10% propanoic acid for 60 min used for size distribution histograms (left, scale bar: 400 nm; right, scale bar: 200 nm).

Table S3. Size, PDI,[a] and zeta-potential of nHAP particles created using different pre-treatment and sonication conditions. Samples were milled for 1 h prior to sonication treatment. Heated samples were subjected to 200 °C for 24 h prior to milling.

Entry	Medium	Time (min)	Heat?[b]	Size (nm)	SD[a]	PDI[a]	SD[a]	Zeta (mV)	SD[a]
S13	5% PA[a]	15	No	641.4	20.21	0.612	0.097	16.6	0.765
S14	5% PA[a]	15	No	252.6	38.7	0.545	0.043	15.2	0.686
S15	5% PA[a]	60	No	701.5	13.55	0.564	0.022	17.1	1.04
S16	5% PA[a]	15	Yes	2146	842.3	0.953	0.082	4.38	1.08
S17	5% PA[a]	60	Yes	2546	3102	0.463	0.468	-1.22	0.999
S18	10% PA[a]	15	No	298.8	11.17	0.443	0.076	12	1.75
S19	10% PA[a]	15	Yes	N/A[c]	N/A[c]	N/A[c]	N/A[c]	6.35	5.62
S20	10% PA[a]	60	No	261.2	30.69	0.389	0.038	8.56	3.04
S21	10% PA[a]	60	Yes	1473	139.9	0.837	0.141	3.78	1.13

a. Abbreviations – standard deviation, SD; polydispersity index, PDI; propanoic acid, PA.
b. Heated samples were subjected to 200 °C for 24 h prior to milling.
c. Instrument could not measure sample. Could be due to agglomeration.

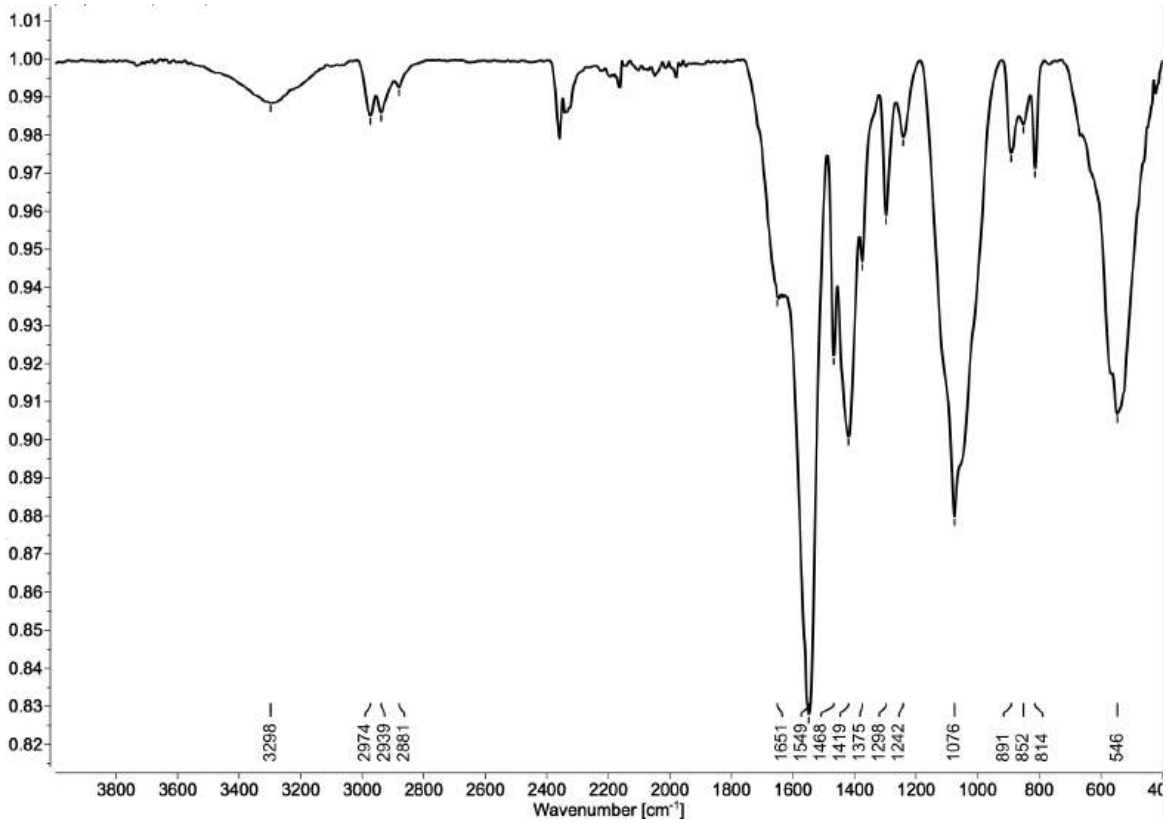

Figure S10. IR spectrum of nHAP prepared with 10% propanoic acid for 15 min.

Table S4. Peak list and assignment of IR spectrum of nHAP prepared with 10% propanoic acid for 15 min.

Wavenumber (cm^{-1})	Functional group	Assignment
3298	O-H	sHAP[1,2]
2974	C-H	Calcium propanoate[3,a]
2939	C-H	Calcium propanoate[3,a]
2881	C-H	Calcium propanoate[3,a]
1651	C=O (amide I)	sHAP[1,4]3/18/25 8:40:00 AM
1549	N-H (amide II)	sHAP[1,4]
1468	CO_3^{2-}	sHAP[1,5] 3/18/25 8:40:00 AM
1419	CO_3^{2-}	sHAP[1,5]
1375	C-H	Calcium propanoate[3,a]
1298	C-O	Calcium propanoate[3,a]
1242	N-H (amide III)	sHAP[1,4]
1076	PO_4^{3-}	sHAP[1,2]
891	CO_3^{2-}	sHAP[1,2]
852	C-H	Calcium propanoate[3,a]
814	C-H	Calcium propanoate[3,a]
546	PO_4^{3-}	sHAP[1,2]

a. While we assign these peaks to calcium propanoate, the data we reference is from sodium propanoate, however peaks should be similar if not identical.

Table S5. Peak list and assignment of XRD diffractogram of nHAP prepared with 10% propanoic acid for 15 min.

2θ, °	Assignment
7.05	Calcium propanoate[6]
11.7	Calcium propanoate[6]
21.1	HAP[7,8] & calcium propanoate[6]
23.4	HAP[7,8,1] & calcium propanoate[6]
29.4	CO_3^{2-} [9] & calcium propanoate[6]
30.6	HAP[7,8,1] & calcium propanoate[6]
31.5	HAP[7,8,1]
34.3	HAP[7,8,1] & calcium propanoate[6]
37.1	CO_3^{2-} [7,9]
41.7	HAP[7,8,1]
42.2	HAP[7,8]
43.5	CO_3^{2-} [9]
45.4	HAP[7,8,1]
46.0	HAP[7,8,1]
48.6	HAP[7,8,1]
49.2	HAP[7,8,1]
50.3	HAP[7,8]
50.8	HAP[7,8,1]
53.6	HAP[7,8,1]
60.9	HAP[8]
64.0	HAP[8,1]
64.9	HAP[8,1]

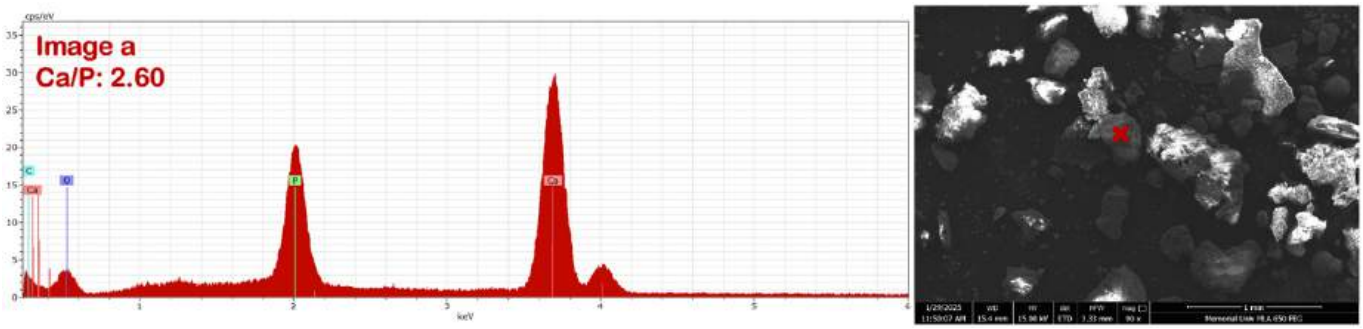

Figure S11. EDX spectrum (left) associated with SEM image “a” (right) of nHAP prepared with 10% propanoic acid for 15 min.

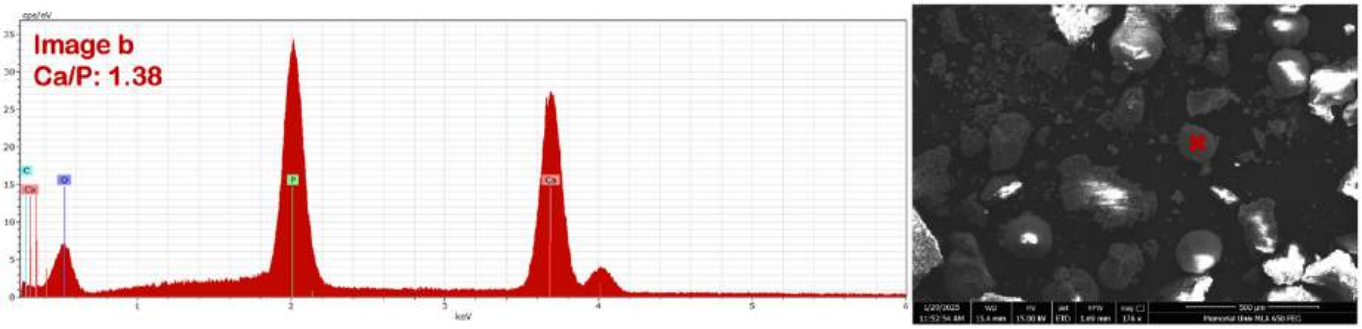

Figure S12. EDX spectrum (left) associated with SEM image “b” (right) of nHAP prepared with 10% propanoic acid for 15 min.

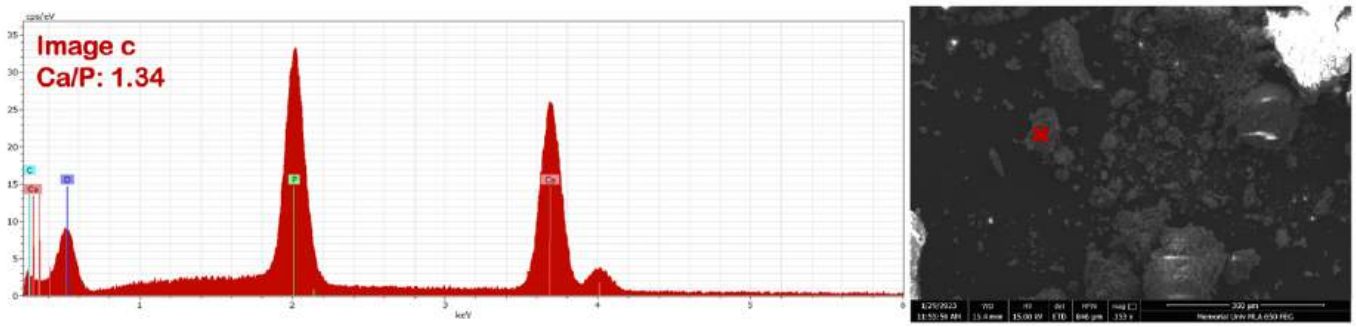

Figure S13. EDX spectrum (left) associated with SEM image “c” (right) of nHAP prepared with 10% propanoic acid for 15 min.

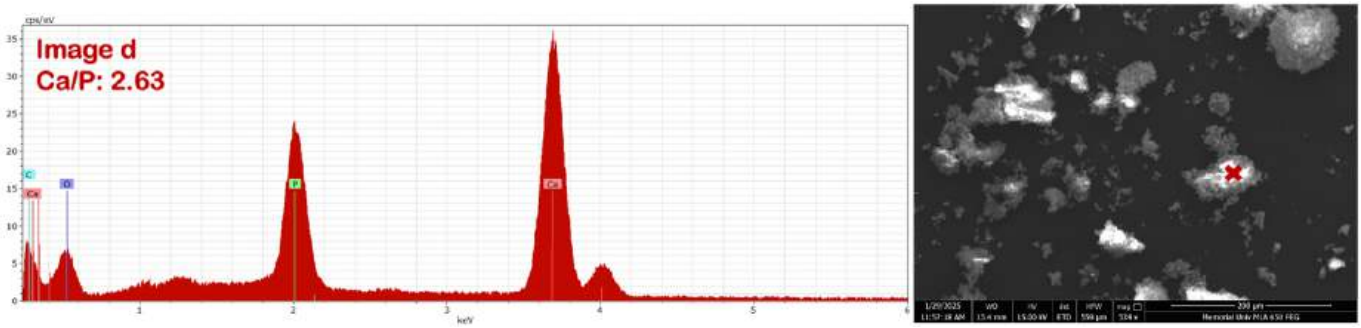

Figure S14. EDX spectrum (left) associated with SEM image “d” (right) of nHAP prepared with 10% propanoic acid for 15 min.

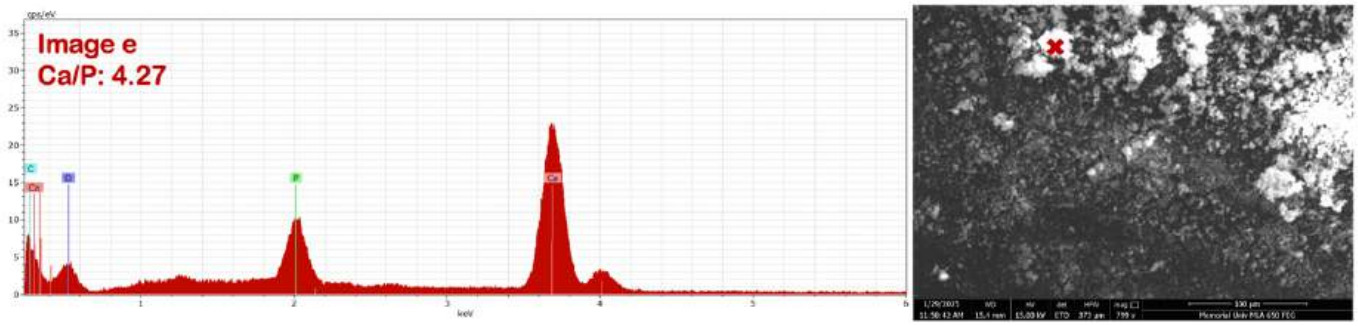

Figure S15. EDX spectrum (left) associated with SEM image “e” (right) of nHAP prepared with 10% propanoic acid for 15 min.

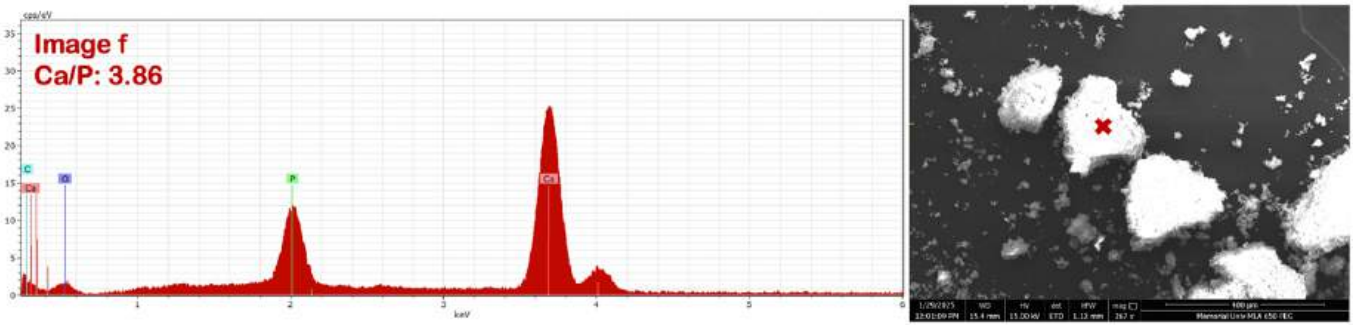

Figure S16. EDX spectrum (left) associated with SEM image “f” (right) of nHAP prepared with 10% propanoic acid for 15 min.

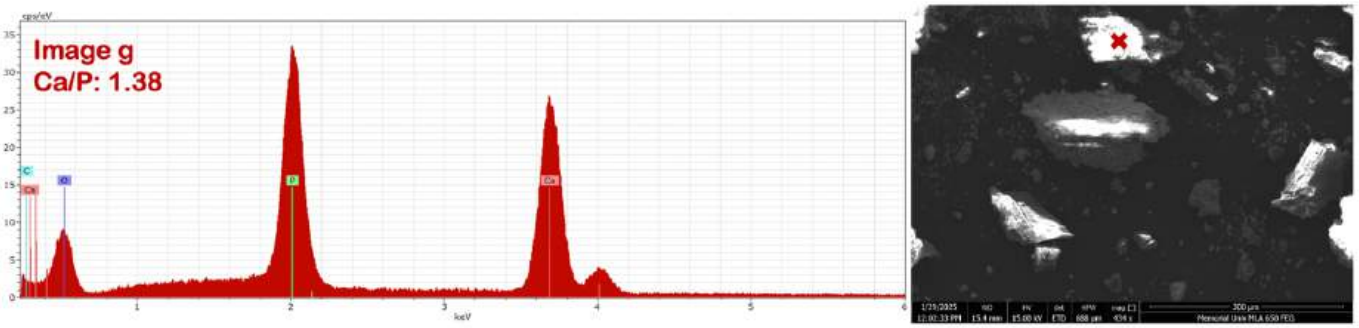

Figure S17. EDX spectrum (left) associated with SEM image “g” (right) of nHAP prepared with 10% propanoic acid for 15 min.

Table S6. Chemical composition of eight EDX spectra from SEM images of nHAP prepared with 10% propanoic acid for 15 min.

Image	C (mass %)	O (mass %)	P (mass %)	Ca (mass %)	P (mol)	Ca (mol)	Ca/P
a (Fig. S11)	5.64	24.3	16.1	54.0	0.519	1.35	2.60
b (Fig. S12)	0.00	33.7	23.8	42.6	0.769	1.06	1.38
c (Fig. S13)	0.120	38.8	22.3	38.8	0.721	0.967	1.34
d (Fig. S14)	12.0	30.0	13.2	44.9	0.426	1.12	2.63
e (Fig. S15)	16.9	29.1	8.28	45.8	0.267	1.14	4.27
f (Fig. S16)	6.78	16.1	12.9	64.3	0.415	1.60	3.86
g (Fig. S17)	0.553	38.5	21.9	39.1	0.708	0.974	1.38
Fig. 7	9.35	30.7	14.0	45.9	0.452	1.15	2.54
Mean	6.41	30.1	16.6	46.9	0.535	1.17	2.50
St. dev	6.14	7.46	5.55	8.49	0.179	0.212	1.13
St. error	2.17	2.64	1.96	3.00	0.0633	0.0749	0.398

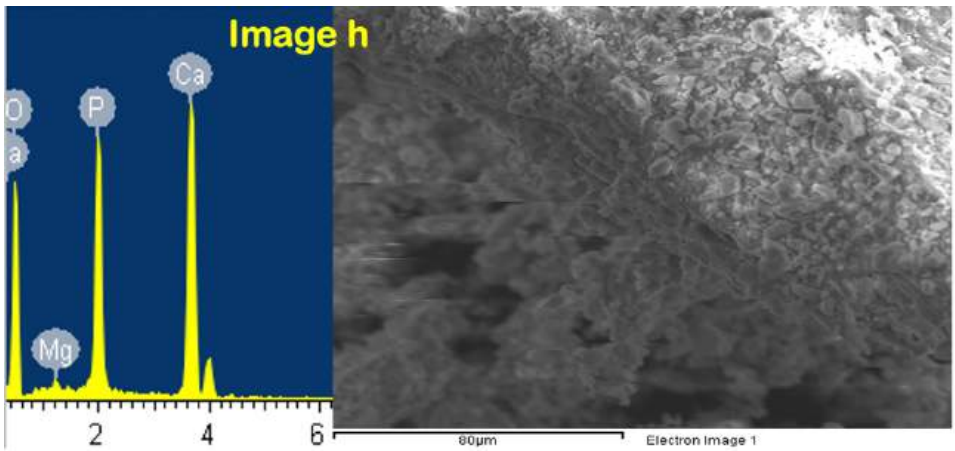

Figure S18. EDX spectrum (left) associated with SEM image “h” (right) of sHAP (original fish bones). Elemental analysis was performed using a Hitachi S-3000N SEM (Hitachi Scientific Instruments, Japan) equipped with EDX detector (INCA x-act, Oxford Instruments) operating in secondary electron/high vacuum mode at 0° sample tilt, 15 kV accelerating voltage and a 15 mm working distance. The samples were immobilized on carbon double-side Pelco 12 mm diameter tabs (Ted Pella Inc.) with the EDX detector focused on the different areas of the sample at several magnifications.

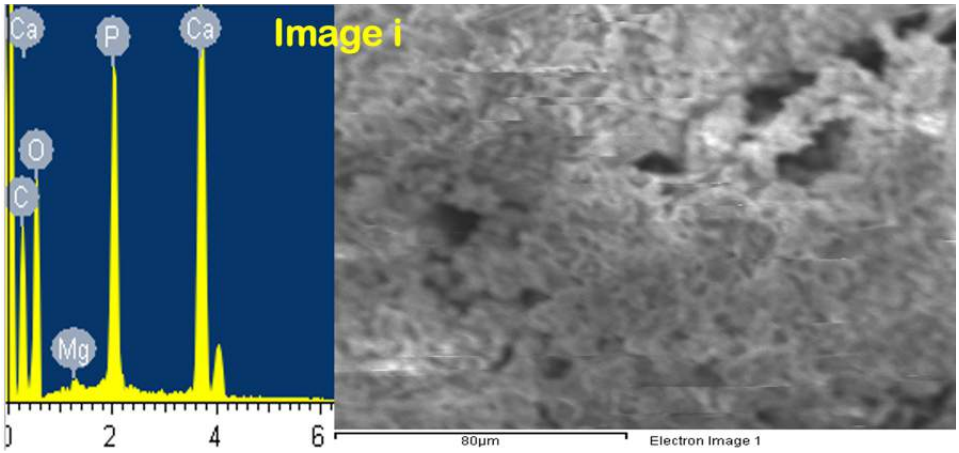

Figure S19. EDX spectrum (left) associated with SEM image "i" (right) of sHAP (original fish bones). Elemental analysis was performed using a Hitachi S-3000N SEM (Hitachi Scientific Instruments, Japan) equipped with EDX detector (INCA x-act, Oxford Instruments) operating in secondary electron/high vacuum mode at 0° sample tilt, 15 kV accelerating voltage and a 15 mm working distance. The samples were immobilized on carbon double-side Pelco 12 mm diameter tabs (Ted Pella Inc.) with the EDX detector focused on the different areas of the sample at several magnifications.

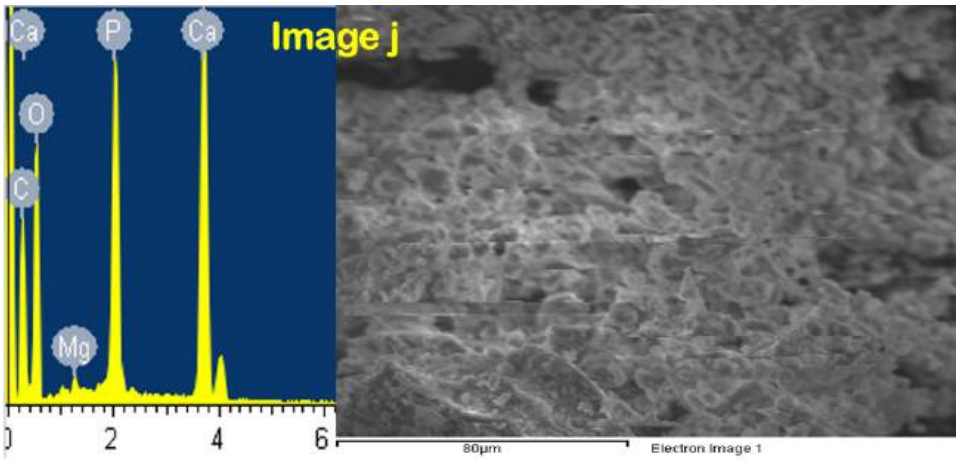

Figure S20. EDX spectrum (left) associated with SEM image "j" (right) of sHAP (original fish bones). Elemental analysis was performed using a Hitachi S-3000N SEM (Hitachi Scientific Instruments, Japan) equipped with EDX detector (INCA x-act, Oxford Instruments) operating in secondary electron/high vacuum mode at 0° sample tilt, 15 kV accelerating voltage and a 15 mm working distance. The samples were immobilized on carbon double-side Pelco 12 mm diameter tabs (Ted Pella Inc.) with the EDX detector focused on the different areas of the sample at several magnifications.

Table S7. Chemical composition of eight EDX spectra from SEM images of sHAP (original fish bones).

Image	C (mass %)	O (mass %)	P (mass %)	Ca (mass %)	P (mol)	Ca (mol)	Ca/P
h (Fig. S18)	27.8	48.1	8.63	15.11	0.279	0.377	1.35
i (Fig. S19)	28.3	44.5	9.32	17.5	0.301	0.435	1.45
j (Fig. S20)	29.3	45.9	9.11	15.31	0.294	0.382	1.30
Mean	28.5	46.2	9.02	16.0	0.291	0.398	1.37
St. dev	0.753	1.80	0.354	1.30	0.0114	0.0324	0.0750
St. error	0.435	1.04	0.204	0.749	0.00659	0.0187	0.0433

Life Cycle Assessment (LCA)

Equations used to Calculate Potentials[10]

i. Acidification potential (I_{AP}) $= \sum AP_i \times m_i$
If not a gas, $AP_i = 0$

ii. Smog formation (I_{SF}) $= \sum SFP_i \times m_i$
$SFP_i = MIR_i / MIR_{ROG}$

iii. Global warming (I_{GW}) $= \sum_i (GWP_i \times m_i) + (X \text{ g } CO_2 \text{ kJ}^{-1} \times Y \text{ kJ})$
Heating liquid $= m \times C_p \times (T_f - 293.15 \text{ K}) \times n$
n = time (h) x 0.5
Oven/furnace
Ramping $= (T_f - 293.15 \text{ K}) \times n \times 3600 \text{ s}$
n = time (h)
Holding $= T_f \times n \times 3600 \text{ s}$
n = time (h)

iv. Toxic inhalation (I_{INHT}) $= \sum_i INHTP_i \times m_i$
$INHTP_i = (C_{i,a} / LC_{50,\,i}) / (C_{tol,\,a} / LC_{50,\,tol})$

v. Toxic ingestion (I_{INGT}) $= \sum_i INGTP_i \times m_i$
$INHTP_i = (C_{i,w} / LD_{50,\,w}) / (C_{tol,\,w} / LD_{50,\,w})$

vi. Persistence (PER)
If PER < weeks = LOW (green)
If PER > weeks, < months = MOD (yellow)
If PER > months = HIGH (red)

vii. Bioaccumulation (ACCU) $= \log(K_{ow})$
If $\log(K_{ow}) < 3.5$ = LOW
If $\log(K_{ow})$ 3.5 – 4.3 = MOD
If $\log(K_{ow}) > 4.3$ = HIGH

viii. Abiotic depletion (I_{AD}) $= \sum_i ADP_i \times m_i$
$ADP_i = ((\text{depletion rate})_i / (\text{reserves})_i) / ((\text{depletion rate})_{ref} / (\text{reserves})_{ref})$

General Assumptions

a. Assumed data for all compounds[10]

Volume air $= 1.00 \times 10^{10} \text{ m}^3$
Volume water $= 7.00 \times 10^{6} \text{ m}^3$
Volume soil $= 9.00 \times 10^{3} \text{ m}^3$
Volume sediment $= 2.00 \times 10^{4} \text{ m}^3$
Density soil $= 1.5 \text{ tonnes m}^{-3}$

Density sediment = 1.5 tonnes m^{-3}
Organic soil fraction = 0.02
Organic fraction sediment = 0.04
Energy consumption = 0.042 g CO_2 kJ^{-1}

b. Oven wattage

The power of various Carbolite-Gero ovens were compared and the average max power and holding power were calculated for an oven that goes to 250 °C (models: AX 30, AX 60, AX 120)[11] and an oven that reaches 1,100 °C (models: ELF 11/16, ELF 11/14, ELF 11/23).[12] The values were normalized to assume all ovens used have an internal volume of 50 L. For a conventional oven reaching 250 °C (drying), the ramping power used for LCA calculations was 11.16 kWh and the holding power was 4.360 kWh. For an oven reaching 1,000 °C (calcination, annealing), the ramping power used for LCA calculations was 1.071 kWh and the holding power was 0.34 kWh. To convert kWh to kJ, the calculated powers were multiplied by 3600 s.

c. Calcination temperature ramp

While many researchers use calcination to isolate hydroxyapatite from wasted biomass, some authors do not include the chosen temperature ramp.[13,14] In these cases, we assume it was 10 °C min^{-1}.

d. Pre-heating conventional oven

For drying purposes, authors do not indicate the time it takes to pre-heat their oven.[13,14,15,16] Therefore, for consistency, we assume for every reference that it takes 15 min to warm up their oven for drying (≤ 250 °C).

Specific Assumptions for References

a. Our method

i. K_{oc} value of enzymes

This is not studied. Enzymes are soluble in water, therefore it should be close to 1. Methanol has a K_{ow} of 0.18 and butanol has a K_{ow} of 6.3. Therefore, we have assigned the K_{ow} of enzymes to be 3 and its log(K_{ow}) would be 0.48. To determine K_{oc}, we multiplied K_{ow} by 0.41 as suggested by Philip Jessop, therefore the value of K_{oc} is 1.23.

ii. H value of enzymes

This is not studied. However, because we assume they have zero volatility, its value has been assigned to H = 1 x 10^{-50}.

iii. Molecular weight of enzymes

MW of proteases has been determined to be in the range of 15 to 30 kDa while the MW of lipases has been determined to be in the range of 19 to 60 kDa, therefore we assigned a MW of 22.5 kDa (2.25 x 10^4 g mol^{-1}) to Neutrase and Alcalase and a MW of 39.5 kDa (3.95 x 10^4 g mol^{-1}) to Lipozyme CALB L.

b. Sharifianjazi et al.[13]

We are assuming 1 L water for boiling.

c. Yamamura et al.[14]

We are assuming 200 g NaOH and 150 g H_2O_2.

Since NaOH is not volatile, its value has been assigned to $H = 1 \times 10^{-50}$.

d. Biazar et al.[15]

Not indicated how much fish bone to start, so we are assuming same amount as our research: ~350 g. Therefore, we will assume treated in 1000 mL water solution with 10 mL NaOH and 10 mL acetone.

It was not indicated how long bones were milled, so we assuming it is the same as our research (1 h).

Since NaOH is not volatile, its value has been assigned to $H = 1 \times 10^{-50}$.

e. Venkatesan et al.[16]

No additional assumptions required.

Example of How CO_2 Emissions are Calculated (Our Process):

For the LCA, we have chosen to use the energy values associated with producing nanohydroxyapatite particles with 10% propanoic acid for 15 min.

a. Pre-treatment

i. Boiling 2 L water for 1 h at 100 °C

$m \times C_p \times (T_f - 293.15\ K) \times n$

$2000\ g \times 4.184\ J\ g^{-1}\ K^{-1} \times (373.15 - 293.15\ K) \times 2$

1,338,880 J = 1,338.880 kJ

b. Enzyme treatment

i. Enzymes in 1 L water at 40 °C for 6 h

$m \times C_p \times (T_f - 293.15\ K) \times n$

$1000\ g \times 4.184\ J\ g^{-1}\ K^{-1} \times (313.15 - 293.15\ K) \times 12$

2,008,320 J = 2,008.320 kJ

c. Nanoparticle synthesis

i. Heating

Oven wattage: 2,000 W

$2{,}000\ W = 2{,}000\ J\ s^{-1}$

2,000 J s^{-1} x 86,400 s = 172,800,000 J = 1,728,000 kJ

ii. Ball-mill

Milling power: 0.25 kWh

E = 0.25 kWh x (3,600 s h^{-1}) = 900 kJ

iii. Ultrasound

Energy provided by ultrasound instrument: 40,876.0 J

d. Totals

Pre-treatment energy (kJ)	Enzyme treatment energy (kJ)	Milling energy (kJ)	US energy (kJ)	Total energy (kJ)	CO_2 emissions (g)
1,337.60	1,003.20	900	40.8760	4,288.076	1.38×10^2

LCA Table Produced

Route	I_{SF}	I_{GW}	I_{INHT}	I_{INGT}	*PER*
Our method	0	138	4.89×10^{-9}	7.74×10^{-1}	NO
Sharifianjazi	0	4.28×10^3	0	0	NO
Yamamura	0	5.49×10^3	5.57×10^{-7}	3.39×10^5	NO
Biazar	1.81	6.90×10^3	7.17	1.70×10^4	NO
Venkatesan	1.42	901	563	5.17×10^4	MOD

Potentials Omitted from LCA Table

I_{AP}, I_{AD}, and bioaccumulation were 0 for all treatments discussed, therefore they have been omitted from the LCA table.

Note: A spreadsheet is also available as part of EDI for consultation.

References

1. S. Boudreau, S. Hrapovic, Y. Liu, A. C. W. Leung, E. Lam and F. M. Kerton, *RSC Sustain.*, 2023, **1**, 1554–1564.
2. P. Kusumawati, P. Triwitono, S. Anggrahini and Y. Pranoto, *Squalen Bul. Marine Fisheries Postharvest Biotechnol.*, 2022, **17**, 1–12.
3. N. O. of D. and Informatics, Sodium propionate, https://webbook.nist.gov/cgi/cbook.cgi?ID=B6010184&Units=SI&Mask=80, (accessed February 3, 2025).
4. P. Surya, A. Nithin, A. Sundaramanickam and M. Sathish, *J. Mech. Behav. Biomed. Mater.*, 2021, **119**, 104501.
5. O. A. Osuchukwu, A. Salihi, I. Abdullahi and D. O. Obada, *Mater. Today: Proceedings*, 2022, **62**, 4182–4187.
6. C. A. O'Connell and D. Dollimore, *Thermochimica Acta*, 2000, **357–358**, 79–87.
7. Ungureanu, D. N., Angelescu, N., Ion, R. M., Stoian, E. V., and Rizescu, C. Z., in *Recent Researches in Communication, Automation, Signal Processing, Nanotechnology, Astronomy and Nuclear Physics*, 2011, pp. 296–301.
8. El Boujaddy, H., Mourabet, M., Abdelhadi, El R., Benanni-Ziatni, M., El Hamri, R., and Abderrahim, T., *J. Mater. Environ. Sci.*, 2016, **7**, 4049–4063.
9. J. N. Murphy, C. M. Schneider, K. Hawboldt and F. M. Kerton, *Matter*, 2020, **3**, 2029–2041.

10. S. M. Mercer, J. Andraos and P. G. Jessop, *J. Chem. Educ.*, 2012, **89**, 215–220.
11. Laboratory Oven AX - Carbolite Gero, https://www.carbolite-gero.com/products/ovens/laboratory-ovens/ax-laboratory-bench-top-oven/, (accessed January 12, 2025).
12. ELF | Carbolite Gero, https://www.carbolite-gero.com/products/chamber-furnaces/laboratory-furnaces/elf/models/, (accessed January 12, 2025).
13. F. Sharifianjazi, A. Esmaeilkhanian, M. Moradi, A. Pakseresht, M. S. Asl, H. Karimi-Maleh, H. W. Jang, M. Shokouhimehr and R. S. Varma, *Mater. Sci. Eng.: B*, 2021, **264**, 114950.
14. H. Yamamura, V. H. P. da Silva, P. L. M. Ruiz, V. Ussui, D. R. R. Lazar, A. C. M. Renno and D. A. Ribeiro, *J. Mech. Behav. Biomed. Mater.*, 2018, **80**, 137–142.
15. E. Biazar, M. Daliri J, S. Heidari K, D. Navayee A, M. Kamalvand, M. Sahebalzamani, F. Royanian, M. Shabankhah and F. Farajpour L, *J. Bioeng. Res.*, 2020, **2**, 10–19.
16. J. Venkatesan, B. Lowe, P. Manivasagan, K.-H. Kang, E. P. Chalisserry, S. Anil, D. G. Kim and S.-K. Kim, *Mater. (Basel)*, 2015, **8**, 5426–5439.