Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Sustainable transparent biotemplate from fish scale waste for ultra-low volume high-sensitive UV-Vis spectroscopy

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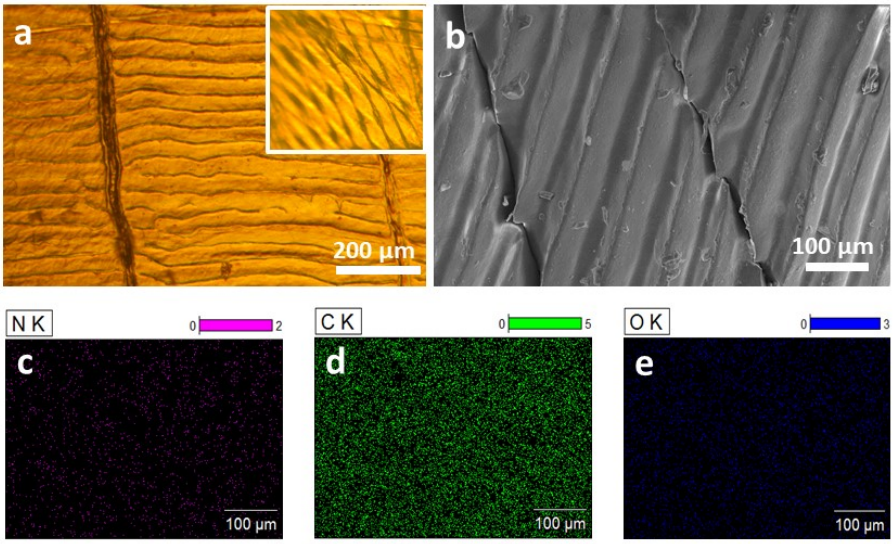
Table S1. Refractive indexes of pristine fish scale and upon processing to transparent biotemplate

Sample No.	1	2	3	4	5	Mean (SD)
Pristine Fish scale	1.669±0.123	1.659±0.112	1.658 0.101	1.664±0.107	1.660± 0.118	**1.662±0.112**
Biotemplate	1.385±0.042	1.383±0.046	1.389±0.052	1.358±0.053	1.387± 0.041	**1.380±0.047**

Refractive index of the biotemplates was measured using Refractometer at 37 °C, by placing ~0.22 ± 0.02 mm thick uniform 1 cm x 1 cm specimens on the test-board, close to the sapphire prism to prevent air bubbles. An average of five measurements were calculated to obtain the mean refractive index.



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Atomic percentage of various elements detected	N	C	O	Ca	P
Biotemplate	22.18	49.24	28.58	0	0

Fig. S1. Detailed structural and compositional characteristics of biotemplate. (a) Optical microscopy images of the biotoemplate revealing the grooved structures. Closer inspection upon trans-sectioned biotemplate revealed super imposed spirally aligned helical structures using optical microscopy (inset of a). (b). HR-SEM showing the smooth biomimetic surface alignment of grooves and ridges (c-f) Elemental mapping analysis of the various elements detected in the biotemplate further revealing the demineralization exposing the inner lamellar hierarchy allowing analyte retainability.

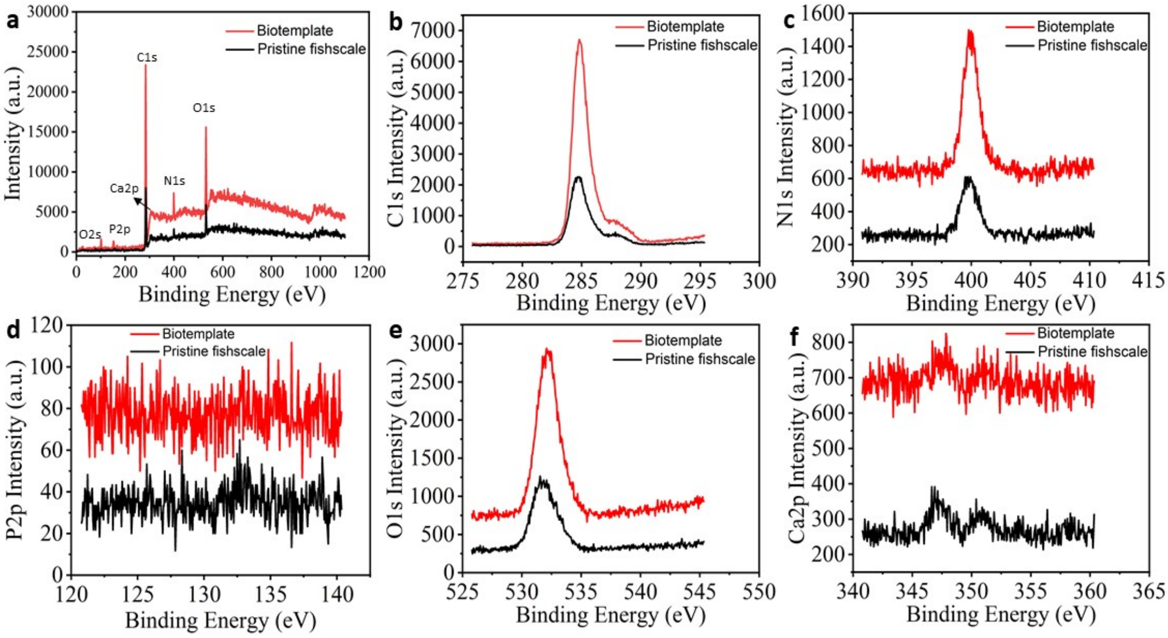

Fig. S2. X-ray photoelectron spectroscopy of the biotemplate and pristine fish scale. (a) Survey scan over a range of binding energies of 0–1400 eV that revealed the presence of , C, O, Ca, N, and P in both the samples. Differences in the core level spectra of the various elements detected (b) C 1s (c) N 1s (d) P 2p (e) O 1s and (f) Ca 2p.

Table S2. Chemical surface composition (%) of various elements detected in the biotemplate versus pristine fish scale using X-ray photoelectron spectroscopy.

Element (%)	N1s	C1s	O1s	Ca2P	P2p
Pristine Fish scale	8.0	75.6	14.4	0.8	1.3
Biotemplate	**4.4**	**77.3**	**17.4**	**0.3**	**0.6**

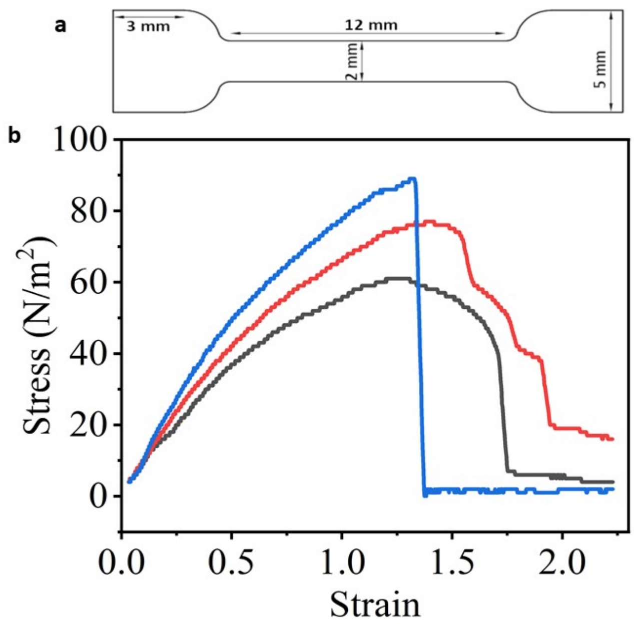

Fig. S3. Mechanical sturdiness of the biotemplate. Tensile strength of the biotemplate performed in triplicate (represented in different colours (b). Orientation and geometry of specimen as dog bone standard (a).

Mechanical strength of the biotemplate is determined as per the American Society for Testing and Materials. Specimen dimensions for the tension testing were shaped to a standard dog bone having gage length (12 mm), diameter (2 mm), radius of fillet (R), width of grip section (5 mm), length of grip section (3 mm), over-all length (21 mm). The tensile strength was measured using Universal Testing Machine (2D Strain Master-LaVision, Germany) with a 100 N load cell and a frame capacity of 1 kN at a crosshead speed of 6 mm/min for at least six independent samples.

Stress–strain responses for the tensile biotemplate revealed an initial portion of relatively nonlinear behaviour and then an increase in slope with increased stress, and the modulus values of the biotemplate was determined to be ~50 $\pm$ 12 MPa. High mechanical sturdiness of the biomatrix is attributed to highly oriented collagen fibres and its interaction with hydroxyapatite accommodating high strength and flexibility to bear the shear and tear while handling the template during spectroscopy measurements.

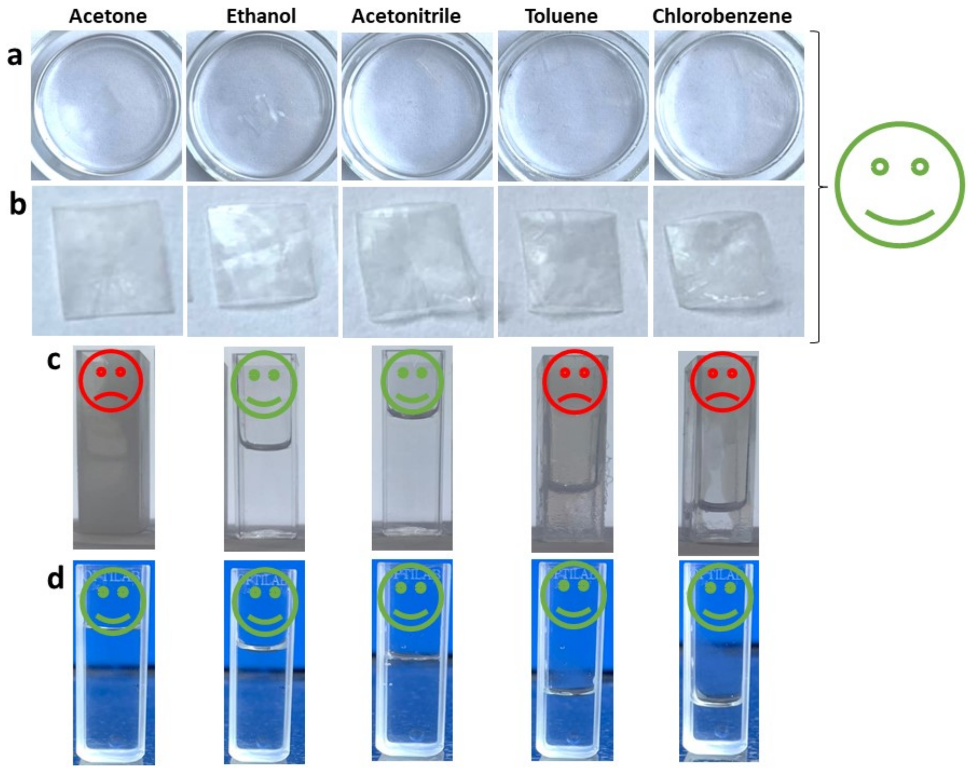

Fig. S4. Solvent stability and compatibility of our biotemplate. Photographs of the biotemplate while (a) and after (b) immersion in different polar and non-polar solvents for 12 hours. Solvent used is indexed in the image. (c) Comparative solvent compatibility of plastic and quartz (d) cuvettes. The biotemplate and quartz cuvette were highly stable in all the solvents without corrosion or deuteration, whereas plastic cuvettes when filled with acetone became opaque (c), and were completely melted when in toluene and chlorobenzene completely damaging the plastic cuvettes (c).

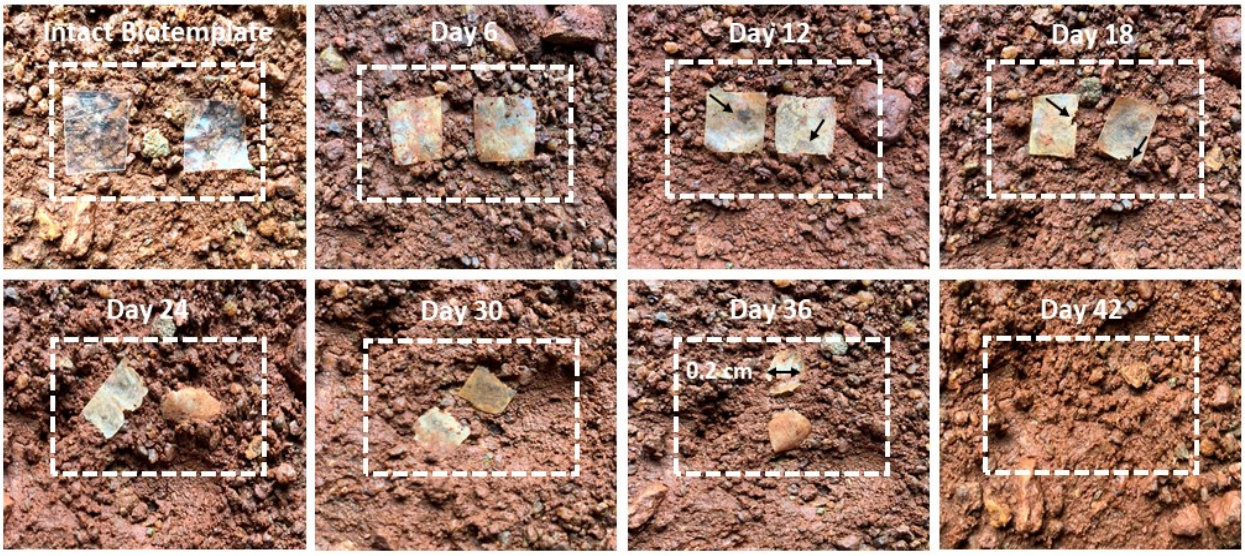

Fig. S5. Biodegradability of the biotemplate. Photographs of the biotemplate degradation process when submerged in various depths of soil. Images representing the day of capture are mentioned in the figure. The biotemplate completely degraded in 42 days. Arrows in day 12 represent the drastic thinning of the biotemplates, arrows in day 18 represent the begun of microbial corrosion from the edges, whereas double headed arrow in the day 36 image represent dramatic reduction in the diameter of the biotemplate. The initial dimension of the intact biotemplate is 1.5 cm x 1.5 cm having a thickness of ± 0.22 0.2 mm.

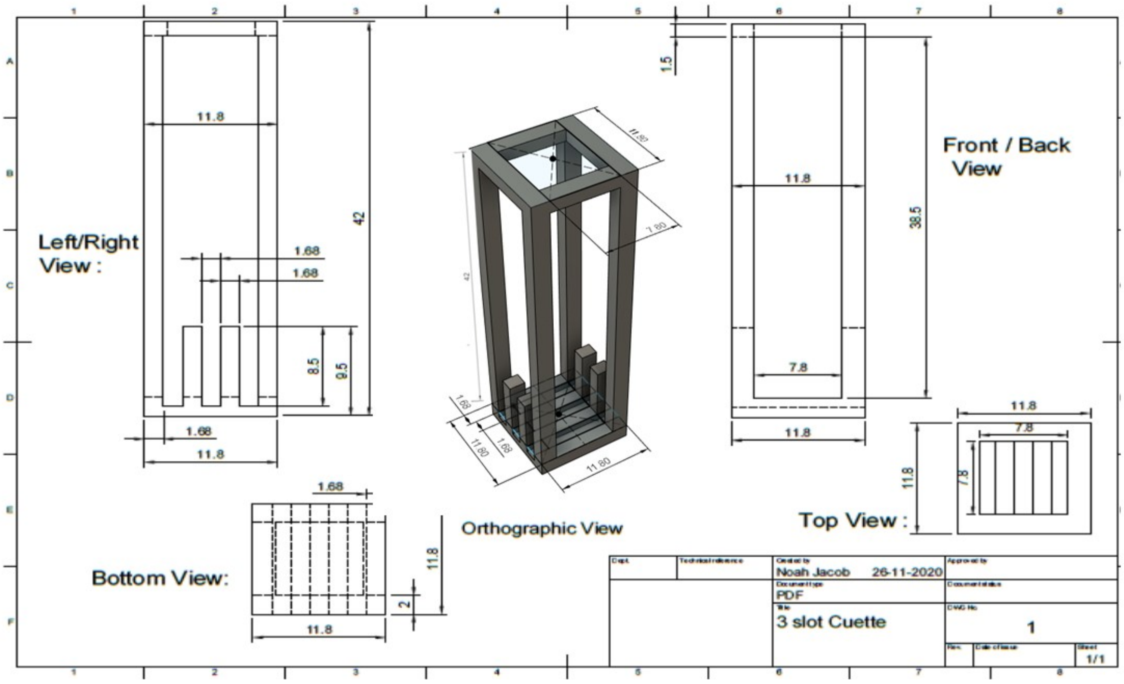

Fig. S6. Design and demonstration of 3D printed cuvette adapter for the biotemplate film for UV-Vis Spectroscopy measurements.

Table S3. Specifications of 3D Printer used

Model and Make	Flash Forge – Finder 2.0
Extruder Diameter	0.4 mm
Max Set Temp of Extruder	240 ^{o}C
Print Speeds	10-100 mm/s
Layer Resolution	0.1 mm - 0.4 mm
Print Resolution	± 0.2 mm

Table S4. Printing details of the biotemplate adapter

Material Used	Polylactic acid (PLA)
Filament diameter	1.75 mm (white)
Extruder temp	220
Print speed	60 mm/s
Layer height	0.18 mm
Perimeter shells	2 shells
Top & bottom solid layers	3 layers
Raft	Yes

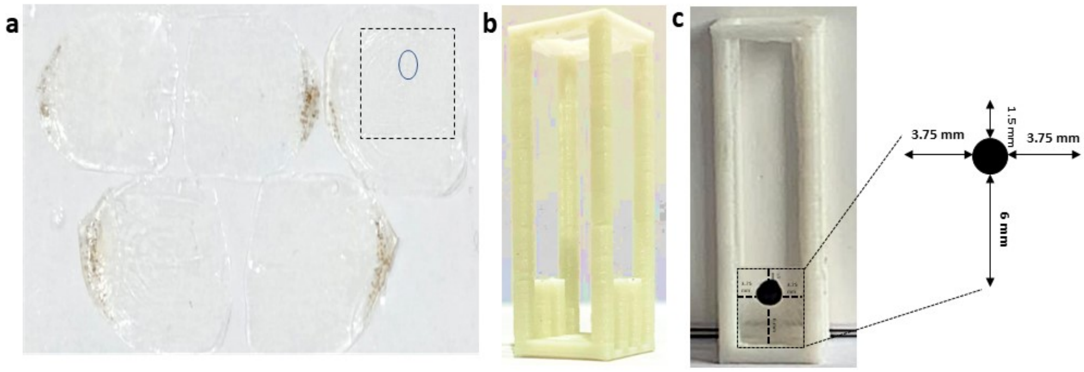

Fig. S7. (a) Square markings illustrating the snipping of 1cm x 1cm biotemplate with circle in the middle represent the analyte drop position matching spectrophotometry light bean path dimensions as provided by the manufacturers. (b) 3D printed cuvette adapter used to support our biotemplate. (c) Image of the drop coloured analyte to show the position of sample loading, along with the details of the sample positioning as per the incidence beam specifications for the spectrophotometer.

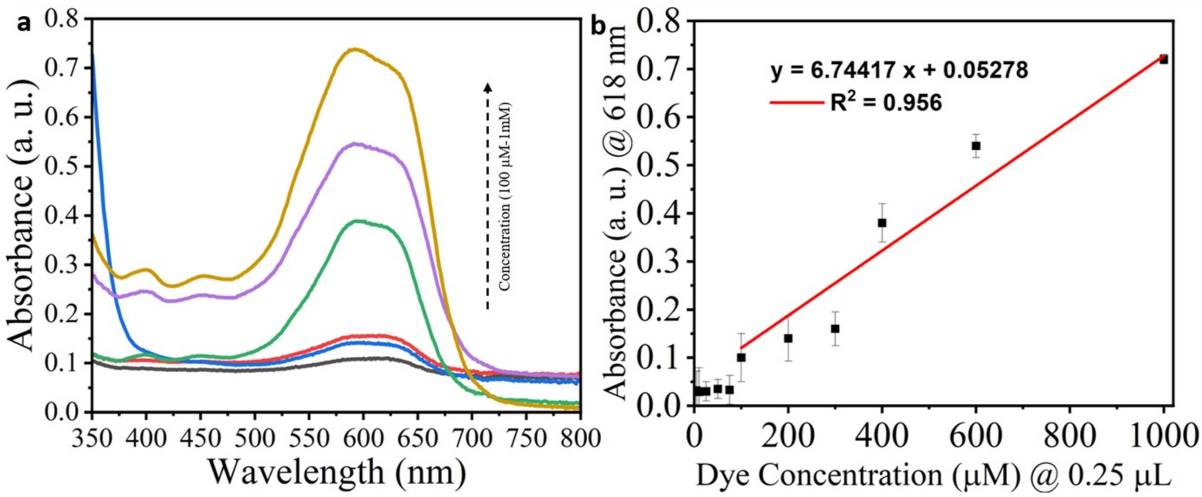

Fig. S8. Validation of on-biotemplate quantitative assessments of Naphthol blue-black at the least 0.25 μL volume. (a) Sensitivity and limit of detection (LOD) (b) analysis using the dye, Naphthol blue-black at constant volume of 0.25 μL and various increasing concentrations of 5 μM, 10 μM, 25 μM, 50 μM, 75 μM, 100 μM, 200 μM, 300 μM, 400 μM, 600 μM and 1000 μM.

Annexure 1. Detailed Cost analysis of the derivation of 3000 biotemplate from fish scale presented in Fig. S1h.

The following cost analysis has been conducted for the preparation of one batch of fish scale derived biotemplates, which yields approximately 3000 biotemplates. The cost analysis for the production of the one time 3D printed UV adapter is also included.

Serial No.	Expenses for:	Cost in Indian Rupees
1	Raw material	20
2	Transportation	66
3	Chemicals	10.8
4	Water	5.74
5	Electricity	0.7
6	Maintenance	0.5
	3D printed UV - Adapter	
7	Material (PLA)	1.6
8	Electricity	0.5
	NET total (1 to 8)	**Rs. 105.84 for ~3000 biotemplates**

1.1. Raw material

The raw material for the production of the bio template was sourced from the local fish markets, where the discarded fish scales are sold at a price of ₹20 per Kg.

1.2. Transportation

To procure and transport the raw material (discarded fish scales) from the markets to the laboratory covering a distance of 15kms and with the price of fuel at 87 ₹/L using a vehicle having an average milage of 20 km/L. The cost of transportation is ₹66.

1.3. Chemicals

1 M NaOH and 10% HCl was used for etching the fish scales incurring a total cost of ₹ 10.8.

1.3.1. NaOH

NaOH at a cost of 714 ₹/Kg was used to make 200 ml of 1M NaOH which required 8 g of NaOH costing ₹5.7.

1.3.2. HCl

HCl at a cost of 255 ₹/L was used to make 200 ml of 10% HCl which required 20 ml of HCl costing ₹5.1

1.4. Water

Water was used in the following categories 1) Municipality water for the prewashing washing of the fish scales 2) The use of packaged potable water for the Milli-Q water system (BRAND). Having considered all the use cases the total water cost is ₹ 5.74

1.4.1 Municipality water

The industrial water tax is ₹5.5 per 1000 Gallon were 1 Gallon = 4.54609 Litres is mentioned; pre washing of the untreated fish scales was done using this water averaging about 20 L (4.4 gal) for 1 Kg of fish scales incurring a cost of ₹0.0242

1.4.2 Packaged Potable water

Potable water is used as feed water to generate Mili-Q water, the cost of potable water is ₹20 per 20 L. Milli-Q water was used for the preparation of chemicals as well as the rinsing of the acid – base treated fish scales to avoid re-mineralization of the biotemplate. A total of 2L was used in total for the rising. The evoQUA water purification system has a recovery rate of 35%, which implies a total of 5.714 L of potable water was used resulting a cost of ₹5.714

1.5. Electricity

The industrial cot of electricity is ₹6.70 per unit (1 kWh) considering the use of electrical Rocker to agitate the fish scales for the chemical etching process and the production of 20L of Milli-Q water resulting in a total cost of ₹ 0.7

1.5.1. Rocker

The power rating of the rocker used is 50W and the rocker was used for a total of 2 hrs during the chemical treatment of the fish scales resulting to a cost of ₹0.67.

1.5.2. Milli-Q system

Power rating of the Milli-Q system is 270 W with a flow rate of 1.8 l/min. Hence to produce the required 2 L of Milli-Q water the system was used for 1.11 min incurring a cost of running of ₹0.034

1.6. Maintenance

The cost maintenance of the milli-Q filter is ₹20,000 per 40,000 L of milli-Q water. Therefore the resulting impact of 2 L would incur a maintenance cost of ₹0.5.

2. 3D printed UV Adapter

2.1. Material: PLA

The UV Adapter Cuvette was printed using Polylactic Acid (PLA) procured at a cost of ₹1100 per Kg. The print used 1.5 g of material costing ₹1.6.

2.2. Electricity

A Flash Forge Finder 3D printer was used to print the UV adapter which has a power rating of 60W which took 1hr to print, incurring a cost of ₹0.5.

Formulae used for the calculations:

Power consumption:

$$Energy\ consumed = power\ rating\ (kW) \times hours\ used\ (h)$$

Note: 1 unit = 1 kW.h

Recovery percentage is defined as

$$Recovery\ \% = \frac{permeate}{Feed\ stock} \times 100$$

Sources of few factors for the cost analysis of biotemplate:

Electricity Tariff: Retail Supply Tariffs 2020-21 - Andhra Pradesh
Water tax: Water Tariffs for Industrial and commercial users, AP- State Government
Milli-Q system: http://www.tenak-labmarket.com/H167/DWN/catalogue_evoqua_de_br.pdf

References

1. X. Zhang, T. Ye, X. Meng, Z. Tian, L. Pang, Y. Han, H. Li, G. Lu, F. Xiu, H. D. Yu, J. Liu and W. Huang, *ACS Nano*, 2020, **4,** 3876-3884.