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Appendix

Preparation of BG11 media

Synechococcus elongatus UTEX 2973 was cultivated in BG-11 medium, which is specifically formulated to support its growth. The composition of BG11 media has been presented in Table A.1. 100 mL aliquots of the prepared media were dispensed into 250 mL flasks sealed with cotton stoppers. These aliquots were then autoclaved at 15 psi for 30 minutes at 121°C. Following autoclaving and cooling, the pH of each aliquot was adjusted.

Table A.1 BG11 media composition.

Component	**Amount (per liter)**
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
Na_2CO_3	0.02 g
Trace metal mix	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L
Trace metals	
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg

Preparation of dry cell weight (DCW) calibration curve of cyanobacteria

1. For the preparation of cyanobacterial dry cell weight (DCW) calibration curve first of all cyanobacterial cultures were grown to exponential phase.
2. 50 mL cell culture aliquotes of different optical density (OD) were pelleted by centrifuging at 5000g for 10 minutes in a 50 mL centrifuge tube.
3. All the pelleted cultures were washed twice by centrifugation using distilled water (DW) to remove the residual media and resuspended in 5 mL of DW.
4. Further the culture aliquotes were transferred in different pre-wieght (W1) and dried alumunium foil cups and placed in an hot air oven at 60 °C for 6h.
5. Then all the cups were weighed (W2) after drying and final weight of biomass were calculated by substracting W2 from W1.
6. At last, a graph was plotted between DCW and OD.

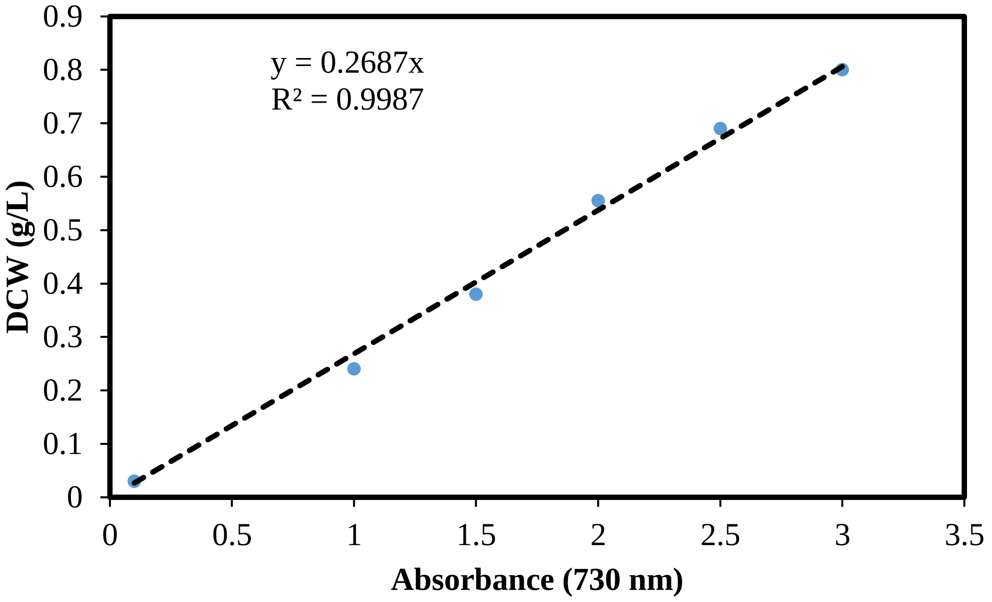

Figure A.1 Dry cell weight calibration curve of *S. elongatus* UTEX 2973 culture.

Protein quantification assay (Bradford method)

The Bradford assay estimates total protein concentration by exploiting the interaction between positively charged amino acids (arginine, lysine, histidine) in proteins and the negatively charged Coomassie dye under acidic conditions. This binding causes a color shift from brown to blue, allowing quantification. Its advantage over the BCA assay is tolerance to low levels of reducing agents and metal chelators, while being hindered by even low concentrations of SDS.

Procedure:

1 Stock solution of BSA, 0.2 mg/ml was prepared.

2 Six test tubes were taken and marked as blank, 1, 2, 3, 4, and 5.

3 In test tubes 1, 2, 3, 4, and 5 stock solution of 50, 100, 150, 200, and 250 µl was added respectively. 2000 µl of sodium phosphate buffer (0.01 M) was added in blank and volume of all tubes was maintained to 2000 µl by adding appropriate volume of buffer.

4 Protein sample was diluted prior to assay in 1:1 ratio (dilution factor =2) by adding buffer. 50 µl from all samples were taken and volume was made up to 2000 µl.

5 5 ml of Bradford reagent was added in all the sample and blank test tubes and incubated in dark for 10 minutes.

6 The absorbance was measured at 595 nm.

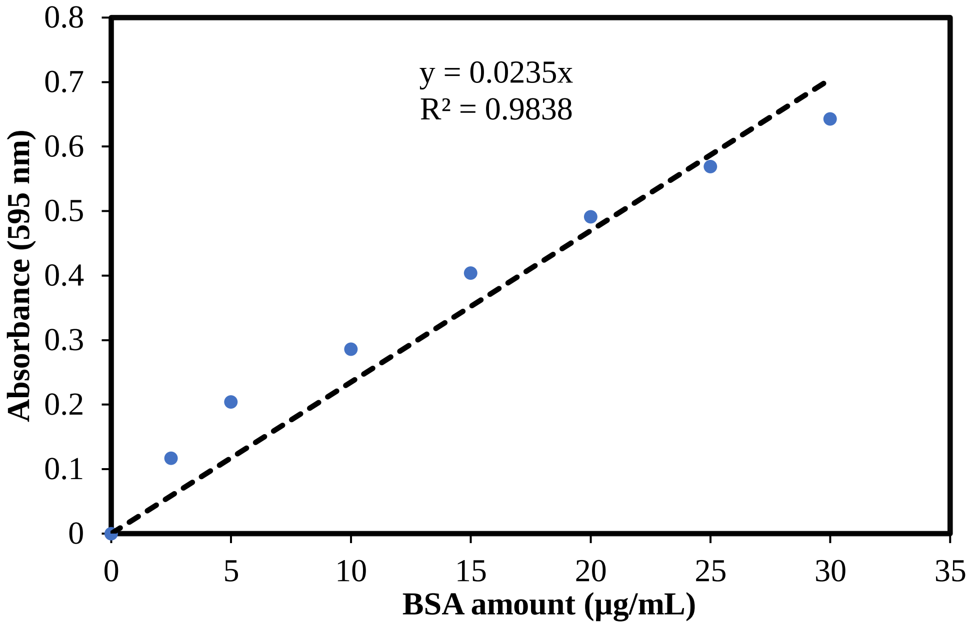

Figure A.2 Protein (BSA) standad calibration curve.

Preparation of isoprene calibration curve

1. Various dilutions of known concentrations of pure isoprene were prepared with air in sealed serum bottles.
2. 100 µl gas samples were taken in a gas-tight syringe from the headspace of the serum bottles and injected into the gas chromatography (GC) system.
3. GC program was set as follows

   Injector temperature: 190 °C

   Detector temperature: 250 °C

   Initial oven temperature: 50 °C

   Hold time: 2 min

   Rt1: 10 °C/min

   Oven temperature 2: 200 °C

   Hold time: 1 min

   Rt2: 2 °C/min

   Oven temperature 3: 210 °C

   Hold time: 2 min

Further, the area of various isoprene dilutions was taken and a graph was plotted against isoprene concentrations.

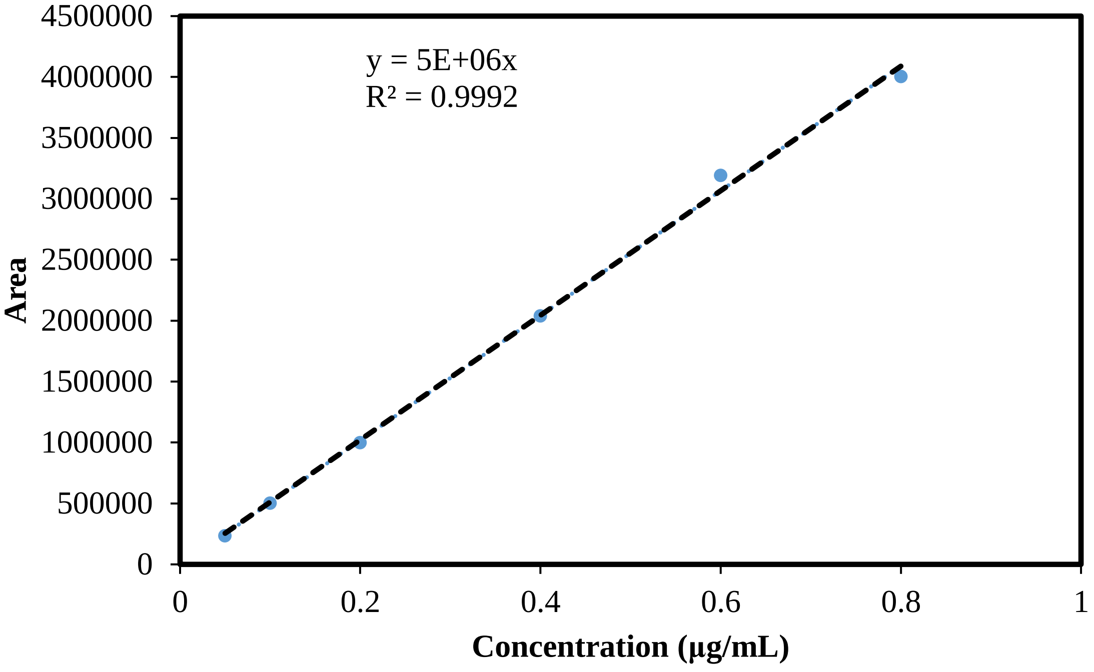

Figure A.3 Isoprene standard calibration curve.

Preparation of plasmid constructs

Preparation of pAM299ISPS

IspS gene was amplified from pBA2SkIkmA2 plasmid by high fidelity DNA polymerase enzyme using IspS-FP and IspS-RP primers. PCR product (*IspS* gene) was run on agarose gel and purified using a Gsure gel extraction kit (GCC Biotech). IspS gene and pAM2991 plasmid were digested with EcoR1 and BamH1 at 37^0 C overnight. Digestion reaction was run on agarose gel and purified using a gel extraction kit. Digested vector pAM2991and insert *IspS* (1:3) were ligated overnight at 16^0 C using T4 DNA ligase. The ligation reaction was used to transform competent *E. coli* DH5α cells. Transformed cells were selected in the presence of 50 µg/mL streptomycin and spectinomycin. Colonies were verified by colony PCR as well as plasmid digestion by EcoR1 and BamH1. Further pAM2991ISPS plasmid was isolated from *E. coli* DH5α using a plasmid extraction kit and transformed into competent *E. coli* HB101 cells for performing triparental conjugation.

Cloning of IDI gene targeted for integration at neutral site III in S. elongatus UTEX 2973 under Ptrc promoter

Addition of NSIII' sequence

Genomic DNA of the *S. elongatus* UTEX 2973 was isolated and used as a template for the amplification of NSIII' (neutral site III upstream region) DNA sequence using NSIII'-FP and NSIII'-RP primers. The amplified NSIII' segment and pBbe1kRFP plasmid were digested with Spe1 restriction enzyme overnight at 37 °C. Digested products were purified using Gsure DNA purification kit and ligated by T4 DNA ligase. The ligated product was transformed in competent *E coli* DH5α cells. Transformed cells were selected by kanamycin (50 µg/ml) and verified by colony PCR as well as plasmid digestion by Spe1 enzyme.

Addition of IDI gene

The *IDI* (isopentenyl diphosphate isomerase) gene was amplified from *E. coli* genome which has NdeI cut site at 5' end and BamHI cut site at 3' end. pBbE1k-RFP-NSIII' plasmid and amplified IDI gene were digested overnight with NdeI and BamH1 restriction enzymes. Digested products were run on 1% agarose gel. Vector and insert were purified using Gsure gel extraction kit. Overnight ligation was done between pBbE1kNSIII' plasmid backbone and *IDI* gene using T4 DNA ligase at 16^0 C. The ligation product was transformed in competent *E. coli* DH5α cells. Integration of *IDI* gene was verified by colony PCR as well as by plasmid digestion.

Addition of NSIII"bom sequence

The NSIII"bom was amplified using fusion (overlap extension) PCR. Further pBbE1k-IDI-NSIII' plasmid and NSIII"bom were digested with PciI and AvrII restriction enzymes. Digested products were run on agarose gel and purified. Purified vector and insert were ligated using T4 DNA ligase. Ligation reaction mixture was used to transform *E. coli* DH5α cells. Verification of insert was done by colony PCR and plasmid digestion by Nde1 restriction enzyme. Further pBb1k-IDI-NSIII plasmid was isolated from the *E. coli* DH5α and transformed in competent *E. coli* HB101 cells for conjugation. A schematic representation of construction and verification of plasmid constructs has been shown in figure A.4.

Genomic DNA isolation from *E. coli*

The protocol procedure involves centrifuging a 1 ml overnight culture of *E. coli* at 5000 g for 5 minutes. The resulting pellets ae subjected to two washes with STE buffer (100 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) followed by another centrifugation at 5000 g for 5 minutes. Subsequently, the pellets are resuspended in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). To this suspension, 100 µl of Tris-saturated phenol (pH 8.0) is added and vortexed for one minute. The solution is then centrifuged at 13,000 g for 5 minutes at 4ºC, resulting in the separation of the aqueous and organic phases. The aqueous phase is carefully transferred to a clean 1.5 ml Eppendorf tube, and its volume is adjusted to 200 µl with TE buffer. Finally, 100 µl of chloroform is added to the aqueous phase. The mixture underwent centrifugation at 13,000 g for 5 minutes at 4ºC, resulting in the separation of the upper layer. Subsequently, chloroform extraction was repeated. A volume of 60 µl from the upper aqueous layer was carefully transferred to a 1.5 ml Eppendorf tube, and the volume was adjusted to 100 µl with TE buffer. To this, 40 µl of TE buffer and 5 µl of RNase (50 mg/ml) were added, followed by incubation at 37ºC for 10 minutes. Another round of chloroform extraction was performed to eliminate RNase, and the resulting aqueous layer was transferred to a clean 1.5 ml Eppendorf tube DNA. For genomic precipitation, 1/10 volume of 3 M Sodium Acetate and two volumes of absolute

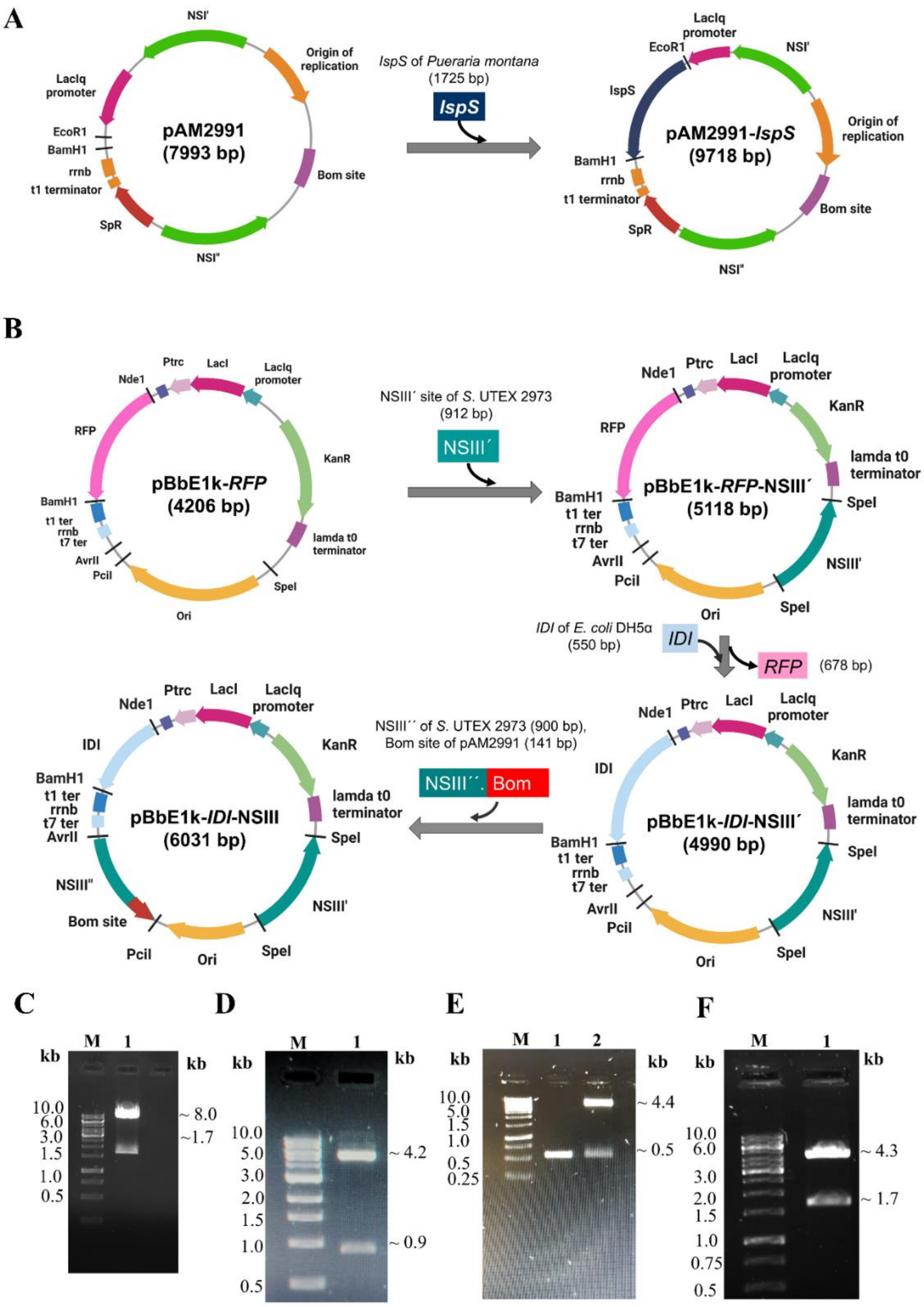

Figure A.4 Plasmid constructs preparation and digestion verification of inserts. (A) Schematic representation of pAM2991-*IspS* construct preparation (B) Scheme of sequential addition of DNA inserts to form BbE1k-*IDI*-NSIII plasmid construct (C) Digestion of pAM299-*IspS* to verify *IspS* insert by EcoRI and BamHI, M- molecular marker, 1- plasmid digest. (D) Digestion of construct pBbE1k-*RFP*-NSIII´ to verify NSIII´ insert by SpeI enzyme, M- molecular marker, 1- plasmid digest. (E) Digestion of construct pBbE1k-*IDI*-NSIII´ to verify *IDI* insert by NdeI and BamHI enzymes, M- molecular marker, 1- *IDI* gene (positive control), 2- plasmid digest. (F) Digestion of construct pBbE1k-*IDI*-NSIII to verify NSIII´´ insert by NdeI (an additional restriction site of NdeI is present in bom sequence), M- molecular marker, 1- plasmid digest.

chilled ethanol (of molecular biology grade) were added to the solution, which was then kept at -20ºC overnight or at -80ºC for two hours. The solution was subsequently centrifuged at 13,000 g for 10 minutes at 4ºC. The liquid above was cautiously poured off, leaving the genomic DNA pellet, which was then rinsed with 70% ethanol. After another round of centrifugation, the pellet was dried thoroughly until all ethanol traces were eliminated from the tube. Subsequently, the pellet was resuspended in 50 µl TE buffer and stored at -20ºC.

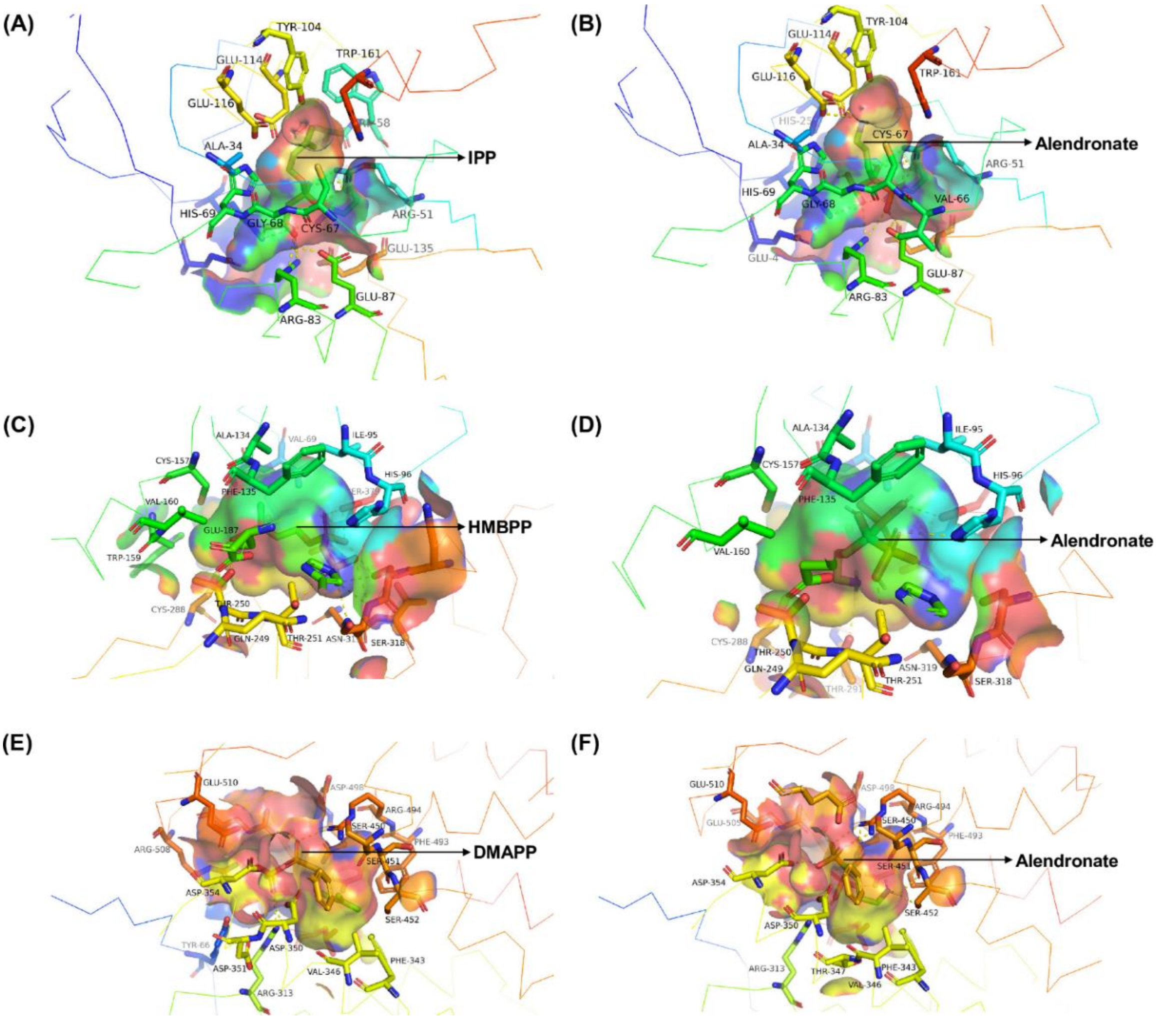

Figure A.5 Lowest energy docked poses of IDI, IspH, and IspS enzymes with their natural substrates and alendronate inhibitor. (A) Docking poses of IDI with IPP and (B) with alendronate (C) Docking pose of IspH with HMBPP and (D) with alendronate (E) Docking poses of IspS with DMAPP and (F) with alendronate. The substrates and alendronate have been labelled and shown as sticks.

Conjugal transfer of plasmid into *Synechococcus elongatus* UTEX 2973

Growth of the cyanobacterial culture (day 1)

1. A freshly grown culture (50 mL) of *S. elongatus* UTEX 2973 was taken. The culture was allowed to grow until reaching an optical density at 750 nm (OD750) within the range of 0.5−1.5, typically taking 1−2 days

Growth of helper and cargo E. coli strains (day 2)

1. A 5 mL volume of LB medium, supplemented with ampicillin (final concentration 100 µg/mL) and chloramphenicol (final concentration 25 µg/mL), was inoculated with *E. coli* HB101 strain containing vectors pRL443 and pRL623 (helper strain). The culture was incubated overnight at 37 °C with continuous shaking at 180 rpm.
2. Also, a 5 mL portion of LB medium, fortified with kanamycin (50 µg/mL), was inoculated with the *E. coli* HB101 culture carrying the cargo vector (pAM2991IspS). The culture was grown overnight at 37 °C with agitation at 180 rpm in a shaking incubator.

Conjugal transfer (tri-parental mating) (day 3)

1. Preparation of the *E. coli* helper and cargo strains for triparental conjugation was performed by centrifuging the overnight cultures of both strains at 3,000 x g for 10 minutes at room temperature. The supernatant was carefully discarded without disturbing the cell pellet.
2. The pellet underwent a washing step by introducing fresh LB medium without antibiotics, using the same volume as the initial culture. Gentle pipetting up and down was employed to resuspend the pellet, emphasizing the avoidance of vortexing to preserve the culture. This washing process was repeated three times to eliminate any residual antibiotics from the overnight culture.

3. After washing the pellet was resuspended in half the volume of LB medium compared to the initial volume. In a 2 mL tube, 450 µL of the helper strain was combined with 450 µL of the cargo strain, and the mixture was set aside at room temperature.

Preparation of the cyanobacterial culture

1. Each conjugation reaction utilized 1 mL of cyanobacterial culture with OD750 within the range of 0.5−1.5.
2. The total volume of cyanobacterial culture required for the reaction underwent centrifugation at 1,500 x g for 10 minutes at room temperature. The supernatant was carefully discarded without disturbing the cell pellet. The pellet was washed by adding fresh BG11 medium of the same initial volume, and subsequent resuspension was carried out by gently pipetting up and down. Vortexing was avoided, and this washing process was repeated three times.
3. An aliquot of the washed cyanobacterial culture (900 µL) was combined with the *E. coli* strains (helper and cargo) (900 µL) in a 2 mL tube.
4. The cultures were mixed by gentle pipetting up and down, avoiding vortexing. The mixture was incubated at RT for 2 hours in the dark.
5. After incubation, the mixture was centrifuged at 1,500 x g for 10 minutes at RT. Subsequently, 1.6 mL of the supernatant was removed, and the pellet was resuspended in the remaining ~200 µL of supernatant.
6. A 0.45 µm membrane filter was placed on an LB-BG11 agar plate lacking antibiotics.
7. Carefully spreading 200 µL of the *E. coli*/cyanobacterial culture mix on the membrane was done using a sterile spreader. The plate was then sealed with paraffin film.

8. The LB-BG11 plate with the membrane was incubated for 24 hours, maintaining membranes with *S. elongatus* UTEX 2973 cultures at 40 °C under a light intensity of 150 μmol photons/m^2/s^1.

Membrane transfer

1. Following a 24-hour period, the membrane was carefully moved to a new BG11 agar plate with the necessary antibiotics for cargo vector selection, using flame-sterilized forceps. The plate was then sealed with paraffin film.
2. The BG11 agar plate was incubated under the specified growth conditions mentioned earlier for *S. elongatus* UTEX 2973 until colonies became visible, typically within a range of 3 to 7 days.

Selection of positive conjugants

1. Using a heat sterile loop, at least two individual colonies were selected from the membrane and streaked them onto a new BG11 agar plate containing appropriate antibiotics.
2. Freshly streaked colonies may still be contaminated with *E. coli* carried over from conjugation (i.e., if small white colonies are evident on the plate), so two or three additional rounds of re-streaking onto fresh BG11 agar plates typically are needed to obtain an axenic cyanobacterial culture.

Analysis of gene expression by semi-quantitative RT-PCR

RNA isolation by RNeasy RNA isolation kit

Procedure:

1. Cyanobacterial cells (*Synechococcus elongatus* UTEX 2973) were grown to 0.5 to 0.7 OD. Cells were harvested by centrifuging at 5000 g for 10 minutes at 4^0 C. Cells were

Snap-freezed for extraction and powdered using a precooled mortar and pestle under liquid nitrogen.

2. Furthermore, RNA was extracted according to the manufacturer's instructions.

First-strand cDNA synthesis:

1. Following components were mixed

Reaction mixture

Component	Volume (µl)
Experimental RNA (up to 5 µg/reaction)	X (2)
Primer [oligo $(dT)_{15}$ 0.5µg/reaction, random primer0.5µg/reaction or gene specific primer 10–20 pmol/reaction	X (1)
Nuclease free water	X (2)
Total volume	5

2. Reaction mixture was heated at 70^0 C for 5 minutes in a preheated heat block. Immediately chilled the tube in ice water, centrifuged for 10 seconds, and kept at the ice until the next step.
3. Reverse transcription reaction mixture (15 µl) was prepared in an ice bath.

Reaction mixture

Component	Volume (µl)
GoScript™ 5X Reaction Buffer	4.0
$MgCl_2$ (final concentration 1.5–5.0mM)	1.2–6.4
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)	1.0
Recombinant RNasin® Ribonuclease Inhibitor (optional)	20 units
GoScript™ Reverse Transcriptase	1.0
Nuclease-Free Water (to a final volume of 15µl)	---
Total volume	15

Reverse transcription mix (15 µl), and RNA and primer mix (5 µl) were combined.

4. Annealing was done at 25^0 C for 5 minutes in a heating block.
5. Extension was done at 42^0 C for 1 h in a heat block.
6. Before proceeding with qPCR, reverse transcriptase was inactivated at 70^0 C for 15 minutes in a heating block.
7. Specific targets in diluted on undiluted cDNA were quantified using Taq PCR master mix.

PCR Amplification of the genes of interest and reference genes (rpoA)

Amplification of IspS using PCR

Reaction mixture

Component	Volume (µl)
Taq master mix	7.5
IspS-rtFP	0.5
IspS-rtRP	0.5
Nuclease free water	5.5
cDNA (as template)	1.0

Reaction conditions

Stage 1: Initial denaturation: 95^0 C for 2 min

Stage 2: denaturation- 95^0 C for 15 sec, annealing- 50^0 C for 20 sec, extension- 72^0 C 10 sec

Stage 3: extension- 72^0 C for 10 min, hold- 4^0 C for infinity.

Amplification of IDI gene using PCR

Reaction mixture

Component	Volume (µl)
Taq master mix	7.5
DXS-rtFP	0.5
DXS-rtRP	0.5
Nuclease free water	5.5
cDNA (as template)	1.0

Reaction conditions:

Stage 1: Initial denaturation: 95^0 C for 2 min

Stage 2: denaturation- 95^0 C for 15 sec, annealing- 55^0 C for 20 sec, extension- 72^0 C 10 sec

Stage 3: extension- 72^0 C for 10 min, hold- 4^0 C for infinity.

Amplification of rpoA using PCR

Reaction mixture

Component	Volume (µl)
Taq master mix	7.5
rpoA-rtFP	0.5
rpoA-rtRP	0.5
Nuclease free water	5.5
cDNA (as template)	1.0

Reaction conditions:

Stage 1: Initial denaturation: 95^0 C for 2 min

Stage 2: denaturation- 95^0 C for 15 sec, annealing- 50^0 C for 20 sec, extension- 72^0 C 10 sec

Stage 3: extension- 72^0 C for 10 min, hold- 4^0 C for infinity.

Reaction conditions:

Stage 1: Initial denaturation: 95^0 C for 2 min

Stage 2: denaturation- 95^0 C for 15 sec, annealing- 47^0 C for 20 sec, extension- 72^0 C 10 sec

Stage 3: extension- 72^0 C for 10 min, hold- 4^0 C for infinity.

All amplified genes were run on 2% agarose gel and visualized under gel Doc.

Analysis of expression of protein using SDS-PAGE

For protein analysis, 10-20 ml of cyanobacterial culture with OD730 of 0.5-1.0 was centrifuged at 1500 g for 10 minutes at 4ºC. The growth medium was discarded without disturbing the pellets. The pellets were resuspended in 500 µl lysis buffer (50 Mm NaH_2PO4, 300 mM NaCl, pH 8.0) or (500 mM Tris-HCl, pH 7.4) or (50 Mm phosphate, 50 mM NaCl, 1 mM EDTA, pH 7.0) and sonicated with probe sonicator five times for 10 s on an ice bath. The lysed solution was centrifuged at 10,000 g for 10 minutes at 4ºC to remove the insoluble fractions. Supernatant which is the cell crude extract can be stored at -20ºC or visualized on gel directly. Meanwhile working solutions for stacking and resolving gels were made which are as follows:

- Solution A (Acrylamide stock solution): 30% (w/v) acrylamide, 0.8% bis-acrylamide (keep the solution on magnetic stirrer until completely dissolved. Be cautious as unpolymerized acrylamide is a skin irritant and a neurotoxin).
- Solution B (4X resolving gel buffer): 1.5 M Tris HCl (pH 8.8), 0.4% SDS.
- Solution C (4X stacking gel buffer): 0.5 M Tris-HCl (pH 6.8), 0.4% SDS.
- 10% Ammonium persulfate (APS).
- Electrophoresis buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS.

- Sample buffer or SDS-PAGE loading buffer: 50 mM Tris-HCl, 20% glycerol, 14.4 mM mercaptoethanol, 2% SDS, 0.1% bromophenol blue.

A 10 to 12% resolving gel (solution A mixed with 1 X solution B, APS, and TEMED) was made which after drying was stacked by 5% stacking gel (solution A mixed with 1 X solution C, APS, and TEMED). Alongside the protein sample was mixed with sample buffer or SDS-PAGE loading buffer and boiled at 100ºC for 5 minutes. After giving sufficient time to polymerize the gel the samples can be directly loaded on the gel or spin down briefly if debris were present. Staining of gel with Coomassie blue requires to make staining and destaining solution as follows:

- Staining solution (1 L): 1 g Coomassie Blue R-250, 450 ml methanol, 450 ml distilled water, and 100 ml glacial acetic acid.
- Destaining solution (1 L): 100 ml methanol, 100 ml glacial acetic acid, and 800 ml distilled water.

After the electrophoresis is complete, the gel is carefully transferred to the staining solution and kept on rocking shaker for 5-10 minutes. The gel was then washed two three times with distilled water and transferred to destaining solution. The gel can be visualised on light box or Chemi-doc.

ANN training data sets containing input and output variables

Light intensity (μmol photon/m^2/s)	**$NaHCO_3$** (mM)	**Temperature** (°C)	**Isoprene Productivity** (μg/L/h)
100	20	41	5.8797
300	20	41	4.7194
100	100	41	23.3412
300	100	41	13.5317
100	60	38	11.9713
300	60	38	5.2897
100	60	44	8.3452
300	60	44	5.5844
200	20	38	5.7916
200	100	38	12.1108
200	20	44	4.5875
200	100	44	16.3319
200	60	41	24.2561
200	60	41	25.4421
200	60	41	23.4322
100	20	41	5.1103
300	20	41	4.4998
100	100	41	23.5417
300	100	41	14.0910
100	60	38	10.6603
300	60	38	5.3890
100	60	44	8.5776
300	60	44	5.6827
200	20	38	5.4693
200	100	38	12.9907
200	20	44	4.3809
200	100	44	16.9133
200	60	41	23.5156
200	60	41	25.0675
200	60	41	23.7124
100	20	41	5.5707
300	20	41	4.3194
100	100	41	23.7145
300	100	41	13.4317
100	60	38	11.1134
300	60	38	5.5897
100	60	44	8.8452
300	60	44	5.7744
200	20	38	5.5516
200	100	38	12.8108
200	20	44	4.2255
200	100	44	16.5719
200	60	41	24.1261
200	60	41	25.2521
200	60	41	23.1321

MATLAB code for ANN-GA based isoprene production process optimization

```
% Solve an Input-Output Fitting problem with a Neural Network
% Script generated by Neural Fitting app
% Created 01-May-2023 23:01:49
% This script assumes these variables are defined:
%   input - input data.
%   target - target data.
x = input';
t = target';
% Choose a Training Function
% For a list of all training functions type: help nntrain
% 'trainlm' is usually fastest.
% 'trainbr' takes longer but may be better for challenging problems.
% 'trainscg' uses less memory. Suitable in low memory situations.
trainFcn = 'trainlm'; % Levenberg-Marquardt backpropagation.
% Create a Fitting Network
%hiddenLayerSize = 5;
net = fitnet([6,6],trainFcn);
% Setup Division of Data for Training, Validation, Testing
net.divideParam.trainRatio = 70/100;
net.divideParam.valRatio = 15/100;
net.divideParam.testRatio = 15/100;
% Train the Network
[net,tr] = train(net,x,t);
% Test the Network
y = net(x);
e = gsubtract(t,y);
performance = perform(net,t,y)
% View the Network
%view(net)
% Plots
% Uncomment these lines to enable various plots.
%figure, plotperform(tr)
%figure, plottrainstate(tr)
%figure, ploterrhist(e)
%figure, plotregression(t,y)
%figure, plotfit(net,x,t)

% Define the input ranges to search over
light_intensity_range = 100:300;
na2hco3_range = 20:100;
temperature_range = 38:44;

fitness_func = @(x) -net(x');
```

```
nvars = 3;
LB = [min(light_intensity_range), min(na2hco3_range),
min(temperature_range)];
UB = [max(light_intensity_range), max(na2hco3_range),
max(temperature_range)];
```

```matlab
% Define the genetic algorithm options
options = optimoptions('ga', ...
    'PopulationSize', 200, ...
    'CrossoverFraction',0.8, ...
    'EliteCount',10, ...
    'MaxGenerations', 1000, ...
    'FunctionTolerance', 1e-4, ...
    'PlotFcn',{@gaplotdistance,@gaplotrange},...
     'MutationFcn',{@mutationadaptfeasible, 0.01},...
    'UseParallel', true);
```

```matlab
% Run the genetic algorithm to find the optimal input values
[optimal_inputs, max_production] = ga(fitness_func, nvars, [], [], [], [],
LB, UB, [], options);
```

```matlab
% Display the optimal input combination and resulting production
fprintf('Optimal Light Intensity: %f\n', optimal_inputs(1));
fprintf('Optimal Na2HCO3: %f\n', optimal_inputs(2));
fprintf('Optimal Temperature: %f\n', optimal_inputs(3));
fprintf('Max Isoprene Productitivity: %f\n', -max_production);
```

MATLAB code for K-fold cross validation of ANN-GA based isoprene production process optimization

```matlab
% Normalise the data
inputN = normalize(input);
targetN = normalize(target);
x = inputN';
t = targetN';


% Choose a Training Function
trainFcn = 'trainlm';  % Levenberg-Marquardt backpropagation.


% Create a Fitting Network
net = fitnet([6,6], trainFcn);


% Set up k-fold Cross-Validation
k = 5; % Number of folds
cv = cvpartition(size(x, 2), 'KFold', k);


% Initialize arrays to store performance metrics for each fold
fold_performance_train = zeros(k, 1);
fold_performance_val = zeros(k, 1);
```

```
fold_r_squared_val = zeros(k, 1);
fold_r_squared_train = zeros(k, 1);
fold_rmse_val = zeros(k, 1);
fold_rmse_train = zeros(k, 1);
fold_mape_val = zeros(k, 1);
fold_mape_train = zeros(k, 1);


for fold = 1:k
    % Get the training and validation indices for this fold
    train_idx = cv.training(fold);
    val_idx = cv.test(fold);

    % Split the data into training and validation sets for this fold
    x_train = x(:, train_idx);
    t_train = t(:, train_idx);
    x_val = x(:, val_idx);
    t_val = t(:, val_idx);

    % Set up the network for training
    net_current = fitnet([6,6], trainFcn);

    % Train the Network for this fold
    [net_current, ~] = train(net_current, x_train, t_train);

    % Test the Network on the validation set
    y_val = net_current(x_val);
    e_val = gsubtract(t_val, y_val);

    % Calculate the performance metrics for this fold (validation set)
    fold_performance_val(fold) = perform(net_current, t_val, y_val);

    % Calculate the performance metrics for this fold (training set)
    y_train = net_current(x_train);
    e_train = gsubtract(t_train, y_train);
    fold_performance_train(fold) = perform(net_current, t_train, y_train);

    % Calculate R-squared for this fold (validation set)
    r_squared_val = 1 - (var(t_val - y_val) / var(t_val));
    fold_r_squared_val(fold) = r_squared_val;

    % Calculate R-squared for this fold (training set)
    r_squared_train = 1 - (var(t_train - y_train) / var(t_train));
    fold_r_squared_train(fold) = r_squared_train;

    % Calculate RMSE for this fold (validation set)
    rmse_val = sqrt(mean(e_val.^2));
    fold_rmse_val(fold) = rmse_val;

    % Calculate RMSE for this fold (training set)
    rmse_train = sqrt(mean(e_train.^2));
```

```
    fold_rmse_train(fold) = rmse_train;

    % Calculate MAPE for this fold (validation set)
    mape_val = mean(abs(e_val ./ t_val)) * 100;
    fold_mape_val(fold) = mape_val;

    % Calculate MAPE for this fold (training set)
    mape_train = mean(abs(e_train ./ t_train)) * 100;
    fold_mape_train(fold) = mape_train;


% Calculate the average performance, R-squared, RMSE, and MAPE across all
folds for validation and training sets
avg_performance_val = mean(fold_performance_val);
avg_performance_train = mean(fold_performance_train);
avg_r_squared_val = mean(fold_r_squared_val);
avg_r_squared_train = mean(fold_r_squared_train);
avg_rmse_val = mean(fold_rmse_val);
avg_rmse_train = mean(fold_rmse_train);
avg_mape_val = mean(fold_mape_val);
avg_mape_train = mean(fold_mape_train);


% Display the average R-squared, RMSE, and MAPE values and individual fold
R-squared, RMSE, and MAPE values for both the validation and training
datasets
disp(['Average R-squared (Validation): ', num2str(avg_r_squared_val)]);
disp(['Average R-squared (Training): ', num2str(avg_r_squared_train)]);
disp(['Individual Fold R-squared (Validation): ',
num2str(fold_r_squared_val')]);
disp(['Individual Fold R-squared (Training): ',
num2str(fold_r_squared_train')]);
disp(['Average RMSE (Validation): ', num2str(avg_rmse_val)]);
disp(['Average RMSE (Training): ', num2str(avg_rmse_train)]);
disp(['Individual Fold RMSE (Validation): ', num2str(fold_rmse_val')]);
disp(['Individual Fold RMSE (Training): ', num2str(fold_rmse_train')]);
% disp(['Average MAPE (Validation): ', num2str(avg_mape_val)]);
% disp(['Average MAPE (Training): ', num2str(avg_mape_train)]);
disp(['Individual Fold MAPE (Validation): ', num2str(fold_mape_val')]);
disp(['Individual Fold MAPE (Training): ', num2str(fold_mape_train')]);
```

List of Publications

- **Indrajeet Yadav**, Akhil Rautela, Agendra Gangwar, Lokesh Wagadre, and Sanjay Kumar* (2023) Enhancement of Isoprene Production in Engineered *Synechococcus elongatus* UTEX 2973 by Metabolic Pathway Inhibition and Machine Learning-Based Optimization Strategy. **Bioresource Technology**. (387), 129677. https://doi.org/10.1016/j.biortech.2023.129677. ***IF 11.4***
- **Indrajeet Yadav**, Akhil Rautela, Agendra Gangwar, Vigya Kesari, Aditya K. Padhi, and Sanjay Kumar* (2023) Geranyl Diphosphate Synthase (CrtE) Inhibition using Alendronate Enhances Isoprene Production in Recombinant *Synechococcus elongatus* UTEX 2973: A Step towards Isoprene Biorefinery" **Fermentation** 9 (3), 217. https://doi.org/10.3390/fermentation9030217. ***IF 3.7***
- **Indrajeet Yadav**, Akhil Rautela, and Sanjay Kumar* (2021) Approaches in the Photosynthetic Production of Sustainable Fuels by Cyanobacteria using Tools of Synthetic Biology. **World Journal of Microbiology and Biotechnology** 37(12):201. ***IF 4.253***
- Vigya Kesari, Sanjay Kumar**, Indrajeet Yadav**, A. Chaterjee, S. Rai, S. Pandey (2021) Ganga River Water Quality Assessment using Combined Approaches: Physico-chemical Parameters and Cyanobacterial Toxicity Detection with Special Reference to Microcystins and Molecular Characterization of Microcystin Synthetase (Mcy) Genes carrying Cyanobacteria. **Environmental Science and Pollution Research**. 29(9):13122-13140. ***IF 5.190***
- Shweta Rawat, Akhil Rautela, **Indrajeet Yadav**, Sibashis Misra, and Sanjay Kumar* (2023) A Comprehensive Review on Enhanced Biohydrogen Production: Pretreatment, applied Strategies, Techno-Economic Assessment and future perspective. **BioEnergy Research**. ***IF 3.8***
- **Indrajeet Yadav,** Akhil Rautela, Shweta Rawat, Agendra Gangwar, and Sanjay Kumar* (2023) Carbon dioxide sequestration and transformation into isoprene using engineered cyanobacteria *Synechococcus elongatus* UTEX 2973 and its techno-economic assessment **(*communicated*)**
- Akhil Rautela, Ajay Kumar Namdeo, **Indrajeet Yadav**, Shweta Rawat Sanjay Kumar* (2022) Recent Advancement in Pretreatment Strategies with Insight into Metabolic Engineering Approaches for Enhanced Biohydrogen Production. **(*Under review*)**
- Agendra Gangwar, Shweta Rawat, Akhil Rautela, **Indrajeet Yadav**, Anushka Singh, and Sanjay Kumar* (2023) Current Advances in Produced Water Treatment Technologies: A perspective of Techno-Economic Analysis and Life Cycle Assessment. **Environment, Development and Sustainability.** 2024 https://doi.org/10.1007/s10668-024-04558-w ***IF 4.9***
- Akhil Rautela, Rishika Chatterjee, **Indrajeet Yadav** and Sanjay Kumar* (2024) A Comprehensive Review on Engineered Microbial Production of Farnesene for Versatile Applications. **Journal of Environmental Chemical Engineering**. 12(2) 112398 ***IF 7.7***

- Akhil Rautela, **Indrajeet Yadav**, Agendra Gangwar, Rishika Chatterjee and Sanjay Kumar* (2024) Photosynthetic production of α-farnesene by engineered *Synechococcus elongatus* UTEX 2973 from carbon dioxide. **Bioresource Technology**. 396, 130432 ***IF 11.4***

Book Chapters

- **Indrajeet Yadav**, Akhil Rautela, Shweta Rawat, Ajay Kumar Namdeo, and Sanjay Kumar* (2022) Metabolic Engineering of Yeast for Advanced Biofuel Production. In Advances in Yeast Biotechnology for Biofuels and Sustainability (pp. 73-97). Elsevier.
- Akhil Rautela, Shweta Rawat**, Indrajeet Yadav,** Agendra Gangwar, and Sanjay Kumar* (2023). Process integration opportunities applied to microalgae biomass production. *Microalgae-Based Systems: Process Integration and Process Intensification Approaches*: 183.
- Jyoti Rani, **Indrajeet Yadav,** Akhil Rautela, Sanjay Kumar (2020) Biovolarization of winery industry waste to produce value-added products In Biovalorisation of Wastes to Renewable Chemicals and Biofuels Eds. Navanietha Rathinam, Rajesh Kumar Sani, Elsevier (ISBN 9780128179529)

Conferences

- **Indrajeet Yadav**, Sanjay Kumar (2022) Photosynthetic production of isoprene by genetically engineered cyanobacteria for biofuel application. International Conference on Sustainable Energy and Environmental Challenges (VII SEEC) organized by Indian Institute of Technology (BHU), Varanasi, India Under the auspices of International Society for Energy, Environment and Sustainability (ISEES) (Dec 16-18, 2022).