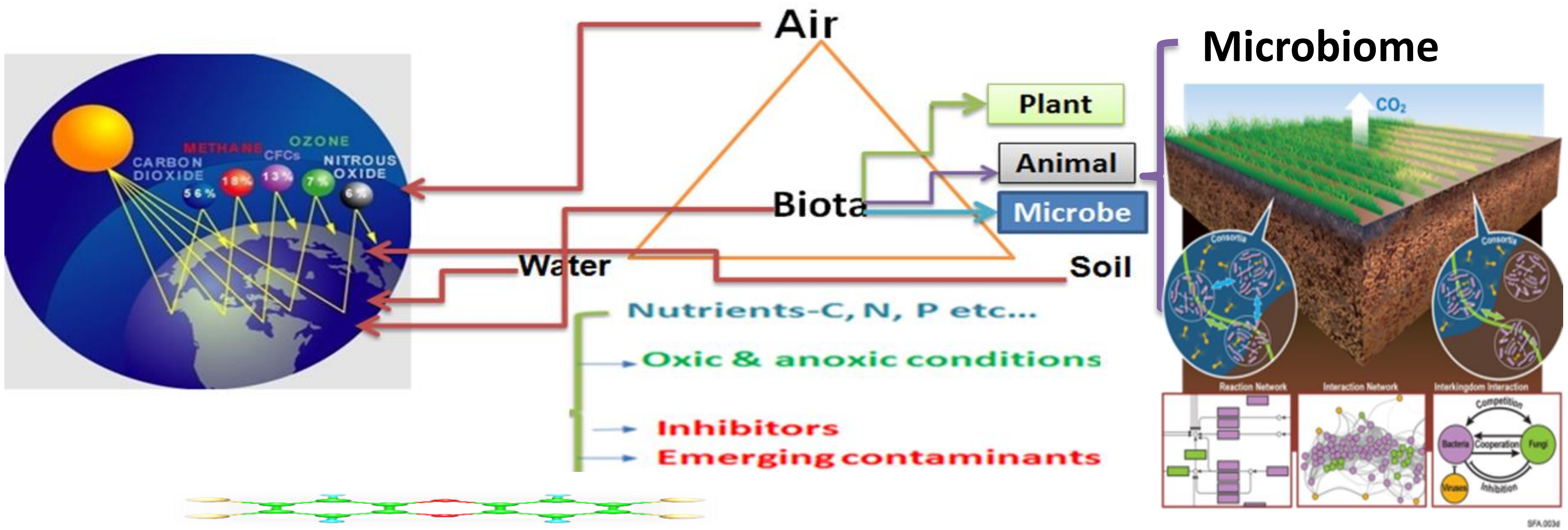
Sequestration of carbon dioxide for production of biofuel and biomaterials by microorganism

Poverty, inequality, protection-Prevention, Detection, Remediation

security for water, food, jobs and climate for sustainability



Microbiome in Climate change and Food

Amity Institute of Microbial Technology
Amity University Noida

Prof. Indu Shekhar Thakur
Amity School of Earth & Environmental Sciences
Amity University Haryana, Manesar, Gurugram
New Delhi-110 067, India

SERB, DST workshop
Date 09/11/2022, 12.00 PM to 12.45 PM

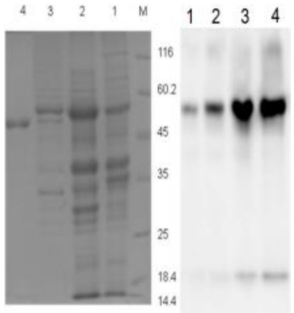
Characterization of carboxylating enzymes and genes for sequestration of carbon dioxide

- Developing bacterial isolates by continuous enrichment and molecular characterization-growth, optimization and production-reactors.
- Enzyme facilitates carbon dioxide fixation-
- Carbonic anhydrase enzyme assay, purification and characterization, optimization.
- Cloning, sequencing and characterization of carbonic anhydrase gene.
- Enzyme fixes carbon dioxide-sequestration process-
- Ribulose-1,5-bisphosphate carboxylase (RuBisCo)- assay, partial purification, Western blot analysis, characterization and optimization.
- SDS-PAGE-MLDI-TOF/MS for RuBisCo.
- Other carboxylating enzymes-
- 2- Dimentional gel electrophoresis MALDI-TOF/MS- & nano drop LC-MS

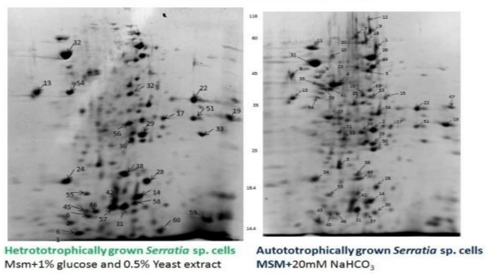
- Whole genome sequencing and metagenomic strategies
- Production of biofuel and biomaterials
- Biodiesel
- Calium-silcon-phosphate
- Exopolysaccharide production
- Bioplastic.

Characterization of carboxylating enzymes and genes for Sequestration of carbon dioxide by *Serratia* sp. IST04

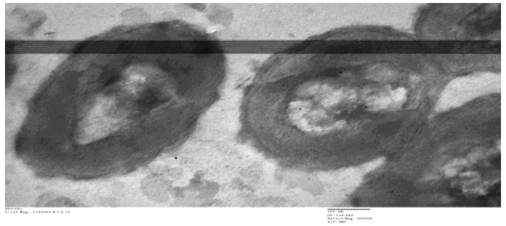
a. Carbon dioxide sequestrating bacterium, identified & molecular characterization.



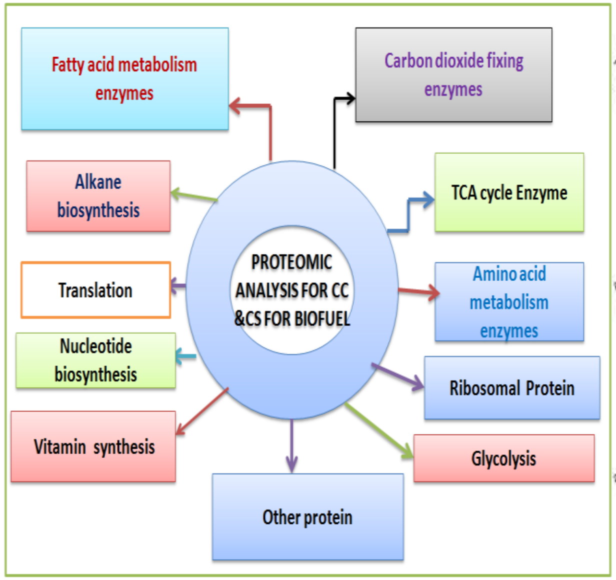
2D gel electrophoresis and MALDI-TOF/MS analysis of Carbon dioxide concentrating *Serratia* sp



b. Sequestration of carbon dioxide for biomass lipids, hydrocarbon, ester.



c. In situ transesterification for biofuel- biodiesel production.



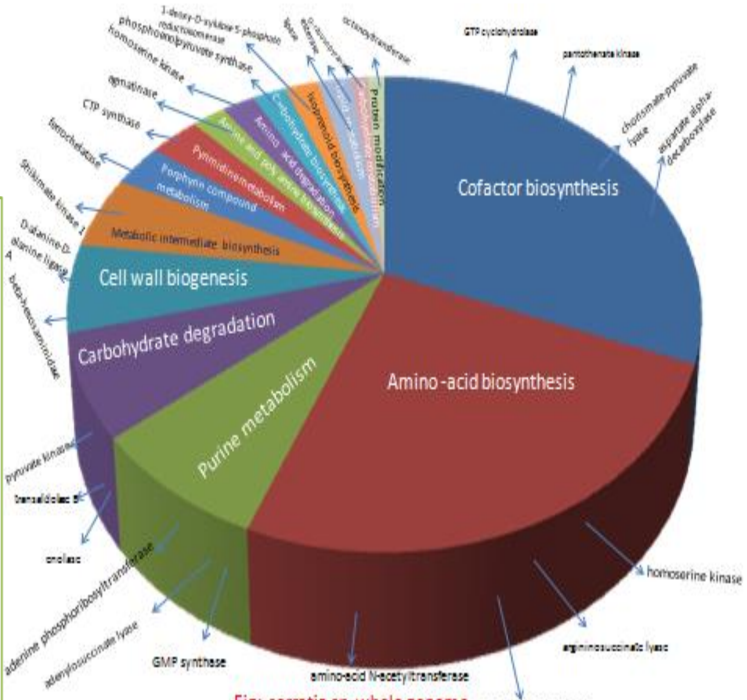


Fig:-serratia sp. whole genome

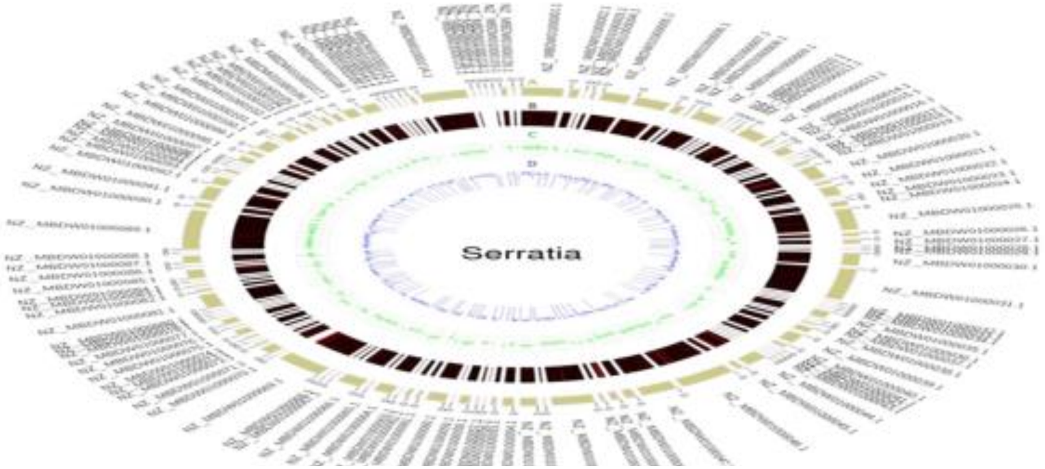
d. Production of exopolysaccharide and polyhydroxyalkalonate.

e. Energy efficient, cost effective less emission biodiesel.

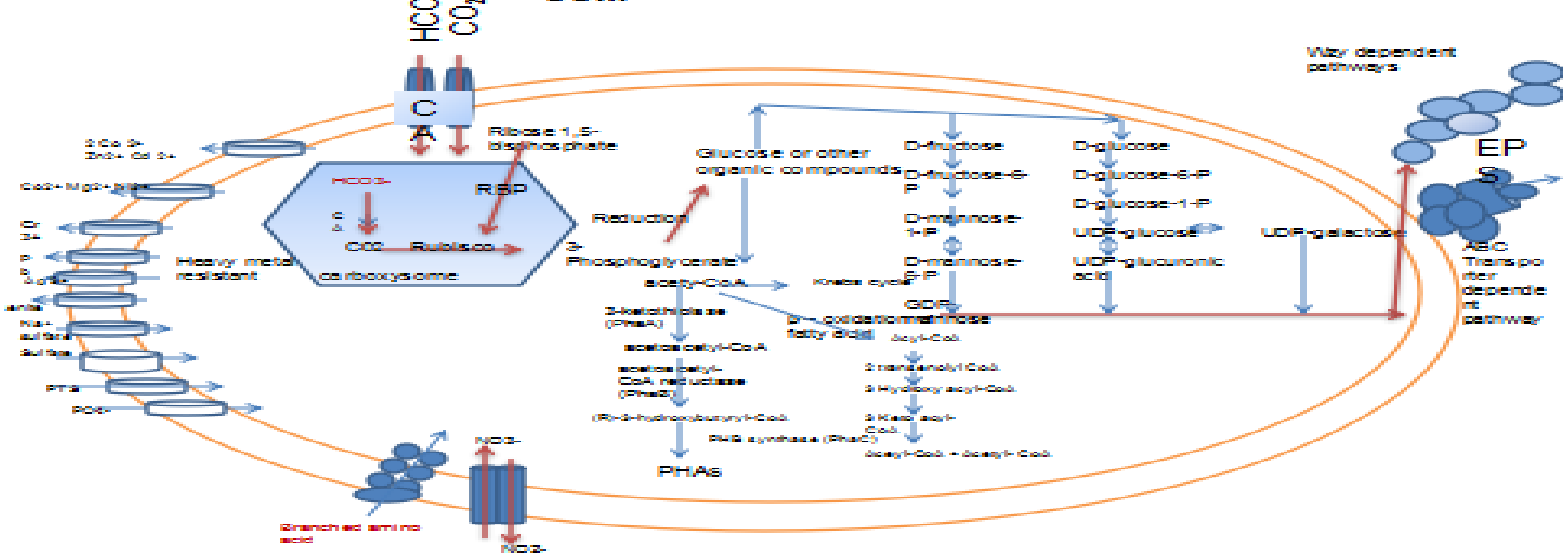
Whole genome sequece analysis of Serratia sp. for biofuel and biorefinery

Table 2: Genome features of the Serratia sp. Strain ISTD04

Genome features	
Total bases (genome)	5,069,140 bp
Total no. of scaffolds	120
Average scaffold size	42,242.833 bp
Scaffold N50	103,262 bp



Physiological apparatus for sequestration of carbon dioxide and production of biomaterials



Kumar, M. et al 2016. Genome announcement 04 (5) e01141-16.

Circos representation of genes compared with the genome for *Serratia sp. ISTD04.*

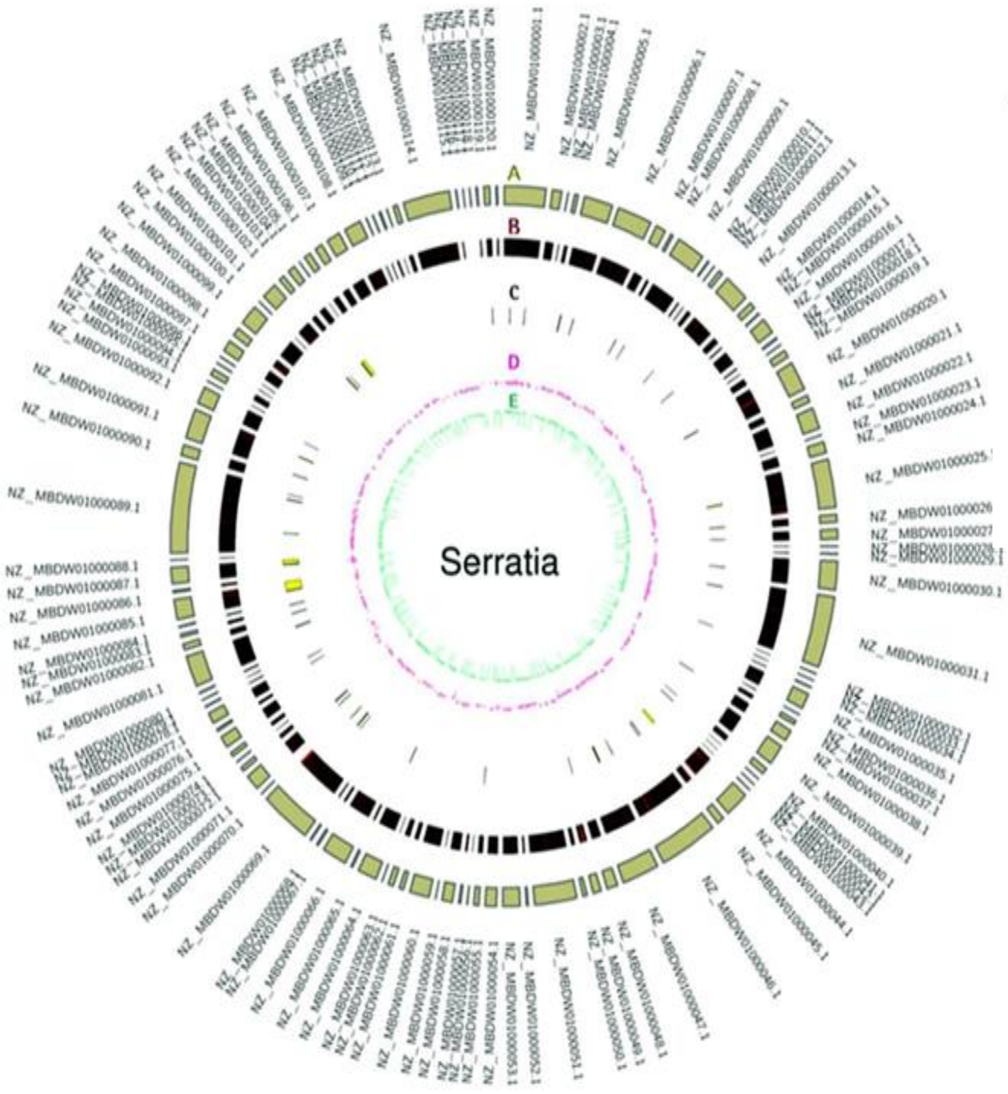
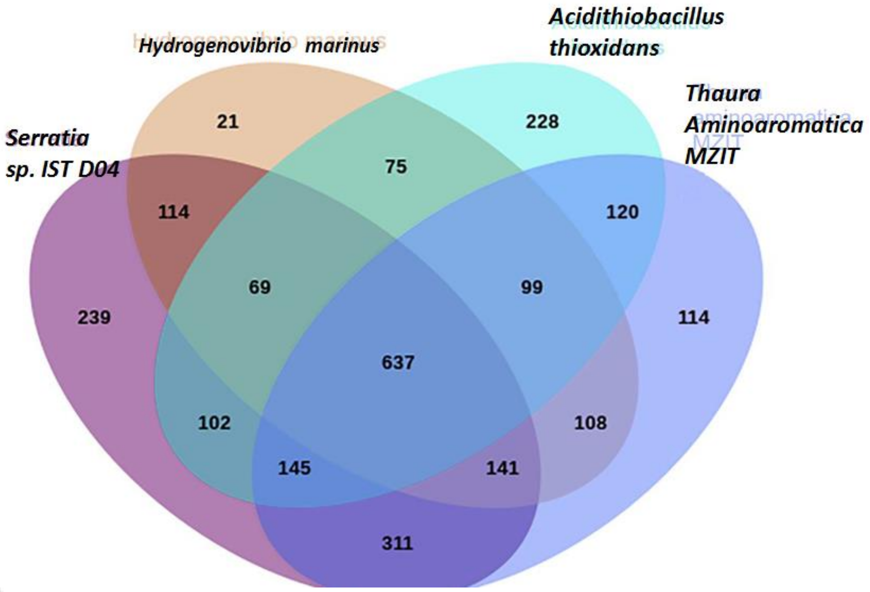
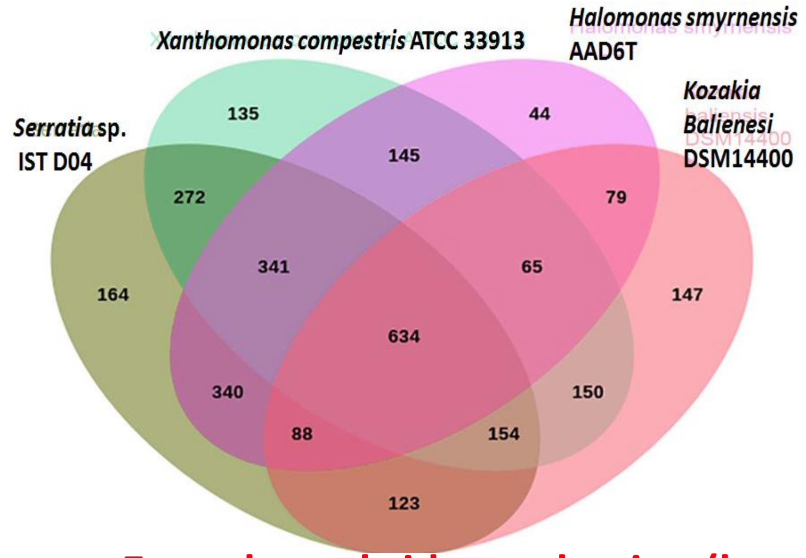


Figure: (Circles from (A) *Serratia* sp. ISTD104 genome, outside; (B) *Serratia* sp. ISTD104 gene; (C) GC skew; (D) GC content

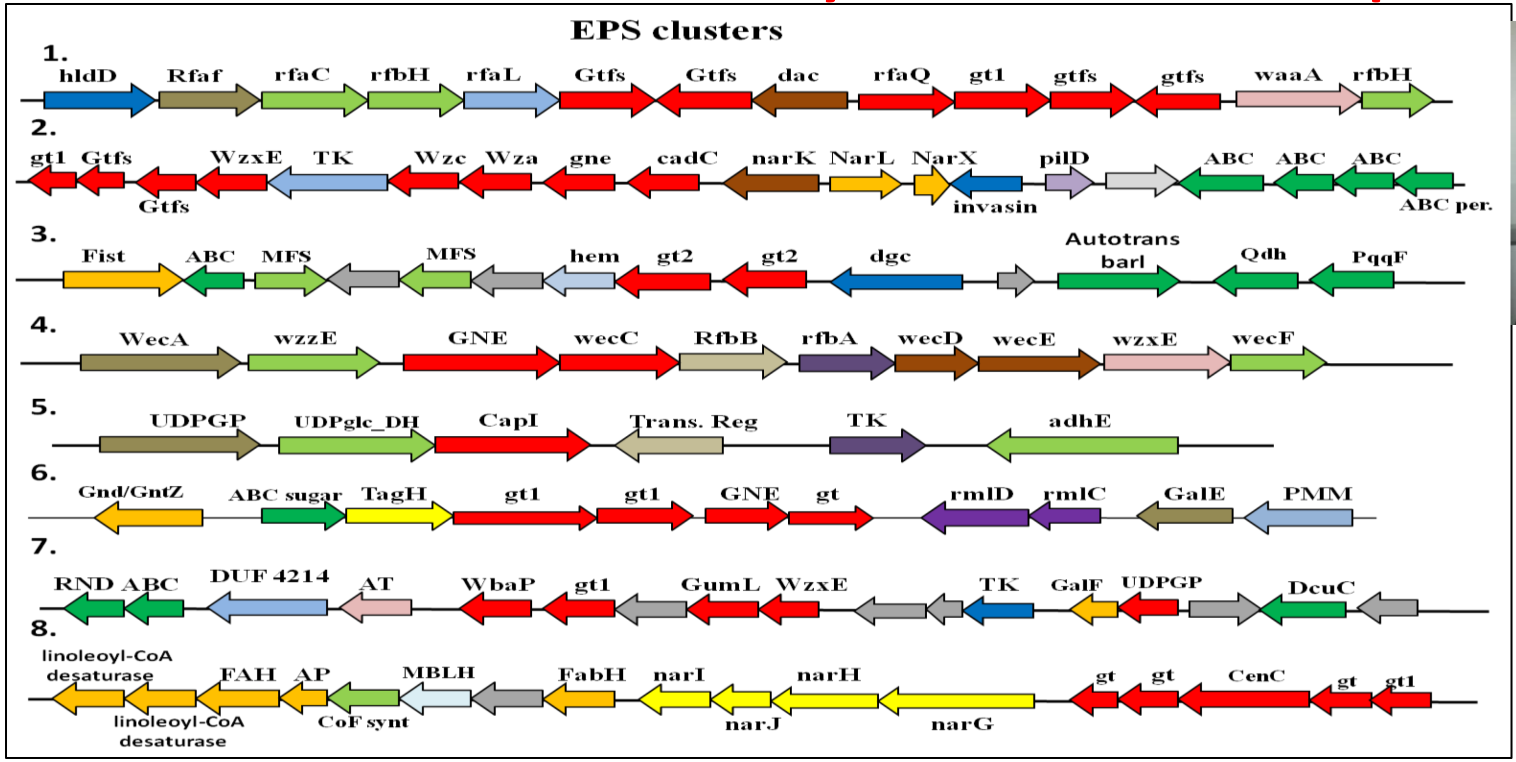


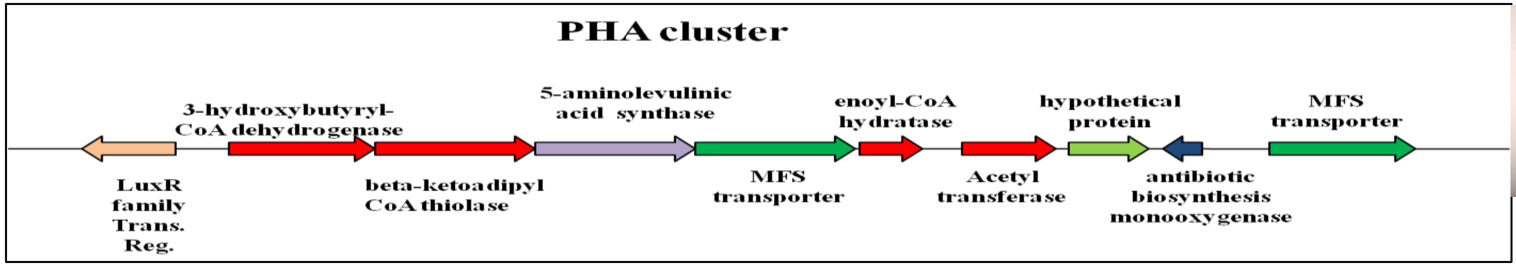
carbon dioxide sequestration (a)



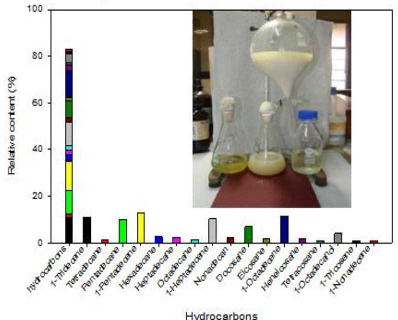
Exopolyssachride production (b

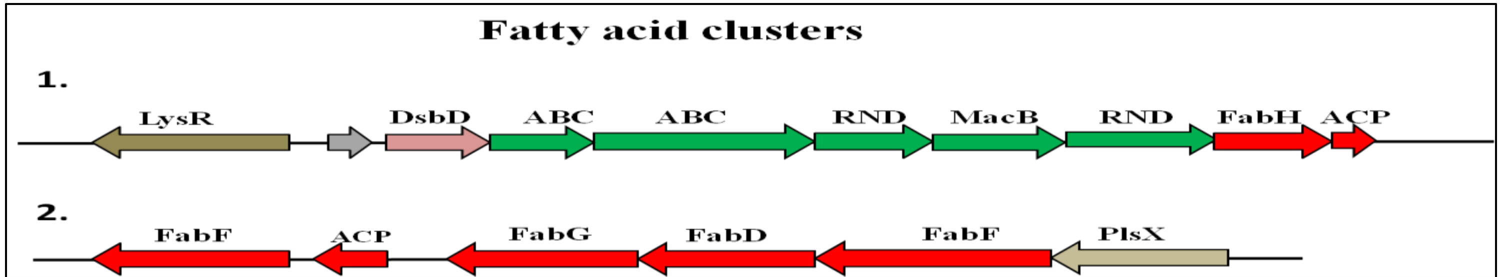
Production of biomaterials by carbon dioxide sequestrating bacteria



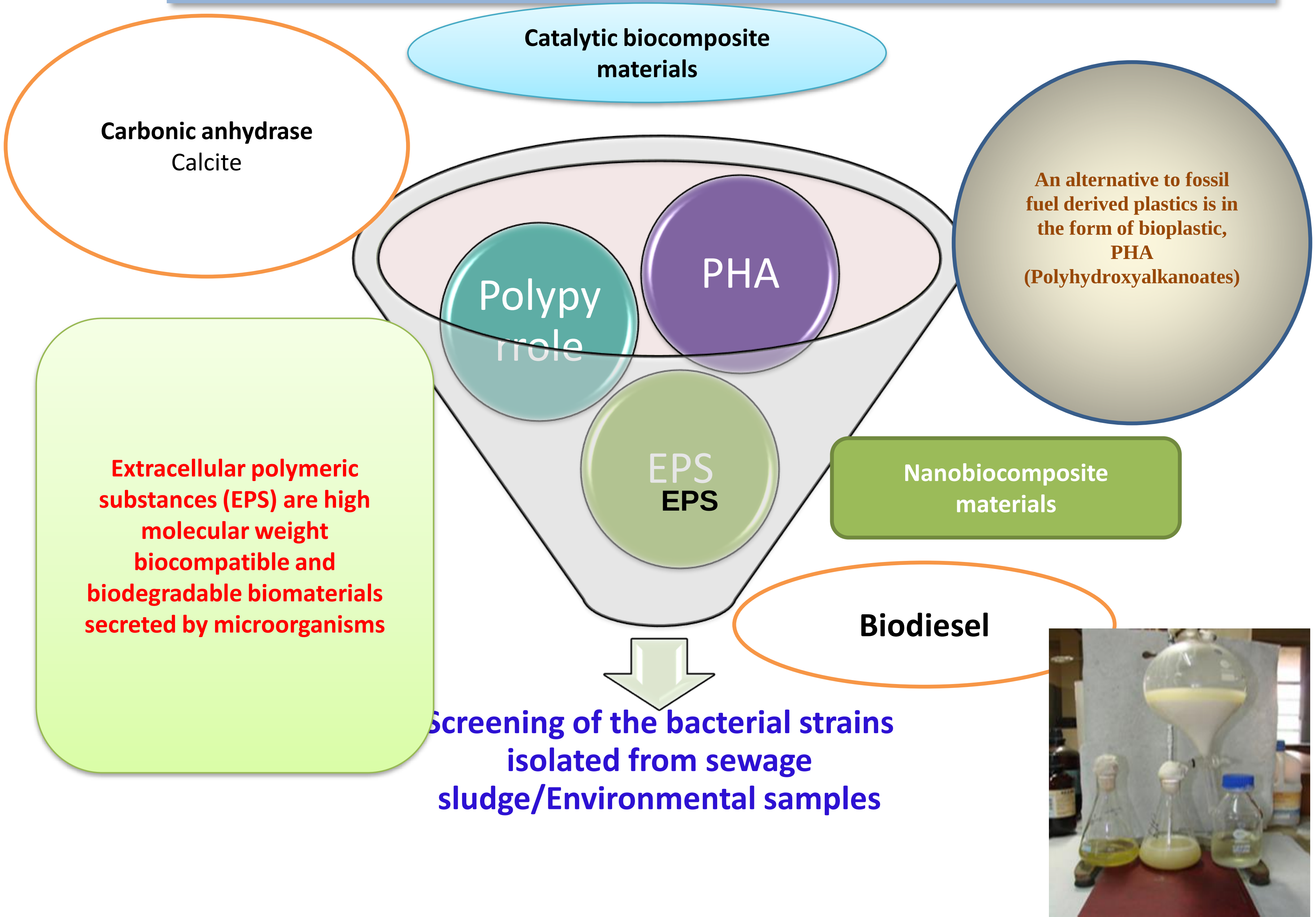


Formation of fatty acid and hydrocarbons by *Serratia* sp. ISTD 04

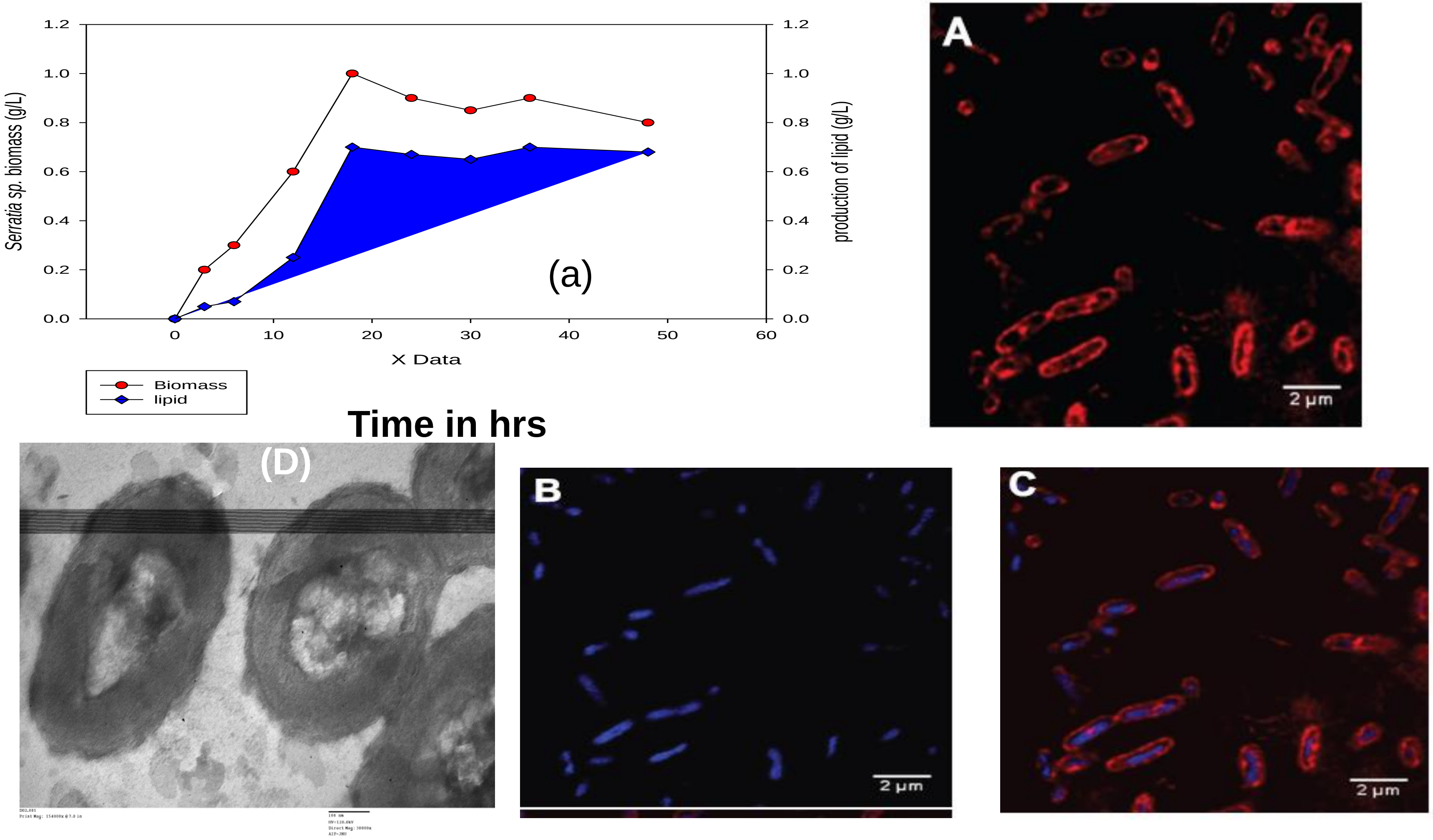




Bio-valorization of Carbon dioxide: Value added products

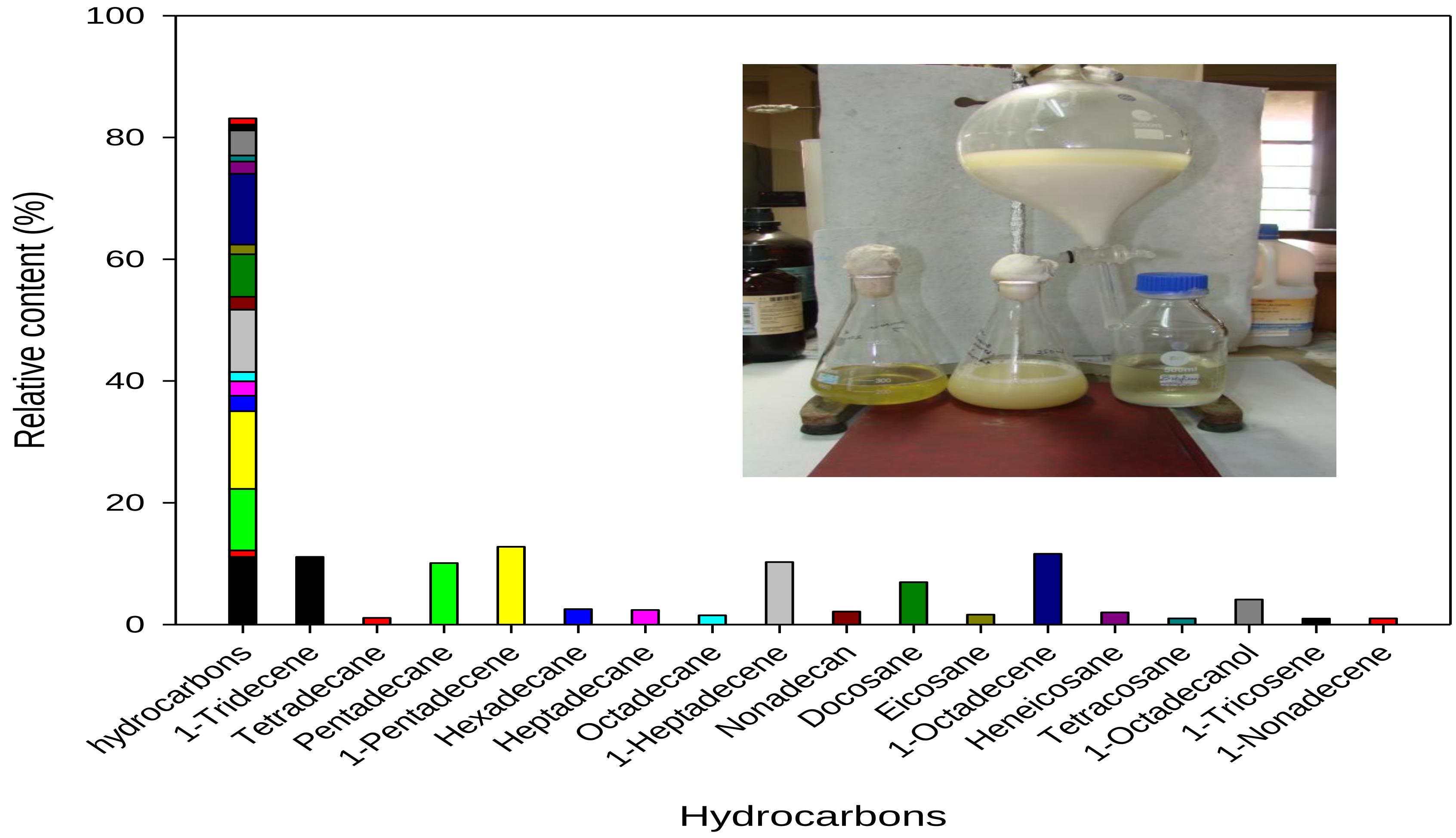


Production of lipid by *Serratia* sp. ISTD04



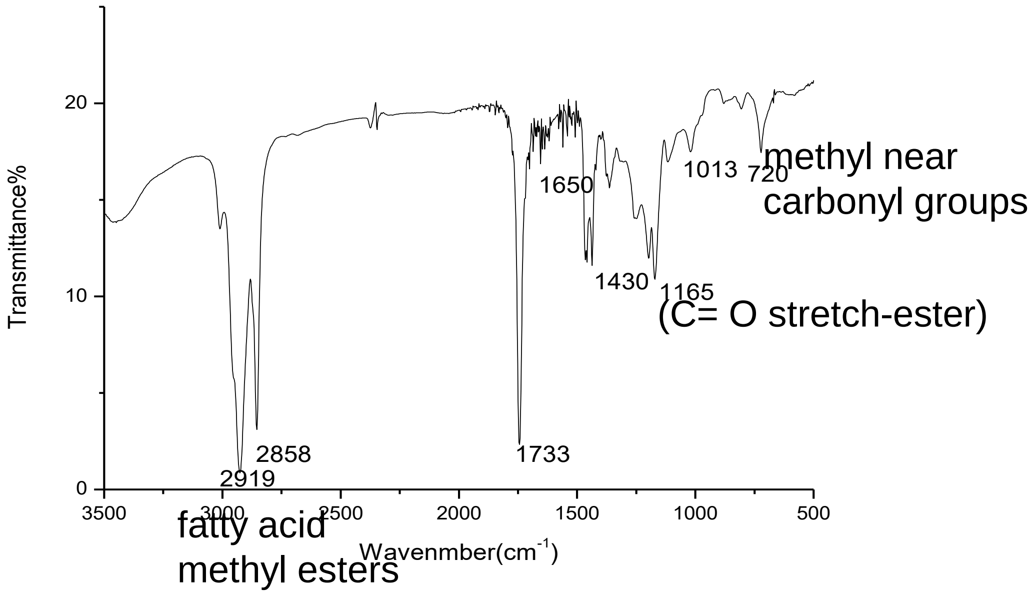
Production of lipids by bacteria, *Serratia* sp. ISTD04, (a); bacterium stained by nile Red (A) ; stained by DAPI (B); and by both DAPI and nile red (C) under confocal microscopy and TEM (D)

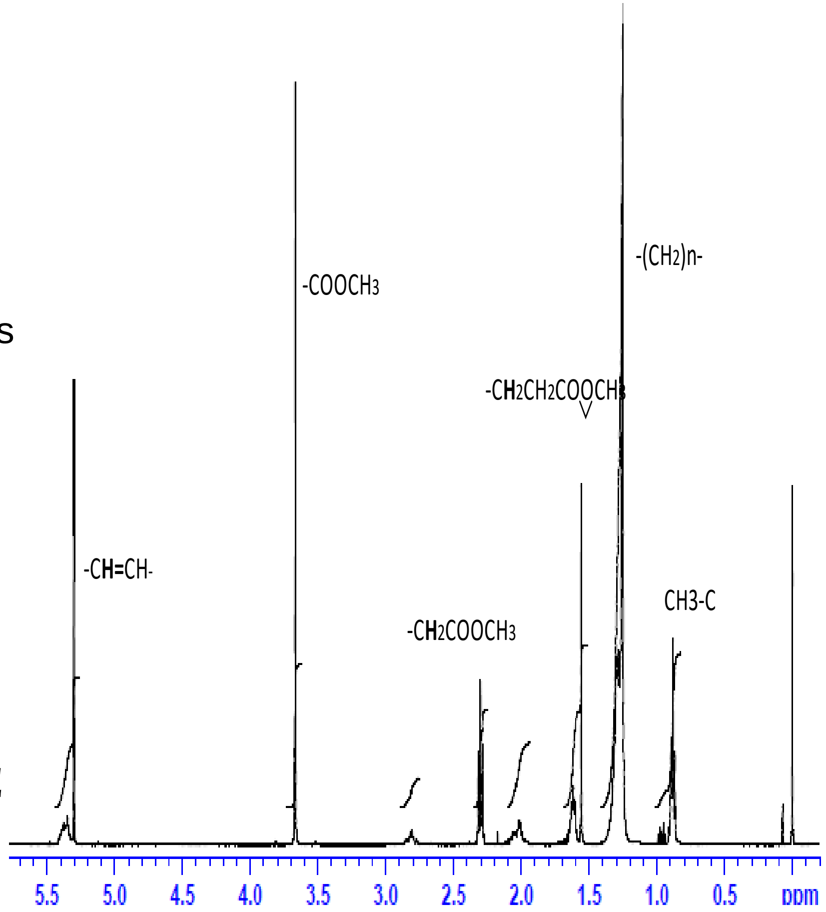
Formation of fatty acid and hydrocarbons by *Serratia* sp. ISTD 04



Most abundant composition of bacterial lipids transesterified with methanol and base catalyst is oliec acid methyl ester

Characterization of biodiesel- FAME produced by *Serratia* sp.





FTIR of FAME (b) 400 MHz ^{1}H NMR spectrum of FAME

1. Olefinic protons –CH=CH– of unsaturated fatty acids.
2. α and β methylene group to ester in FAME.
3. Protons of methyl group of ester.
4. FTIR and NMR spectrum also reflects the conversion of triacylglycerols to methyl esters
5. Unsaturated fatty acids-55%, Saturated fatty acids -45%.

Energy balance of Biodiesel Production

Energy balance per ton biodiesel produced:

$\sum$ Energy output $- \sum$ **energy input (for 1 ton biodiesel)**

Energy input → | Process | Energy output →

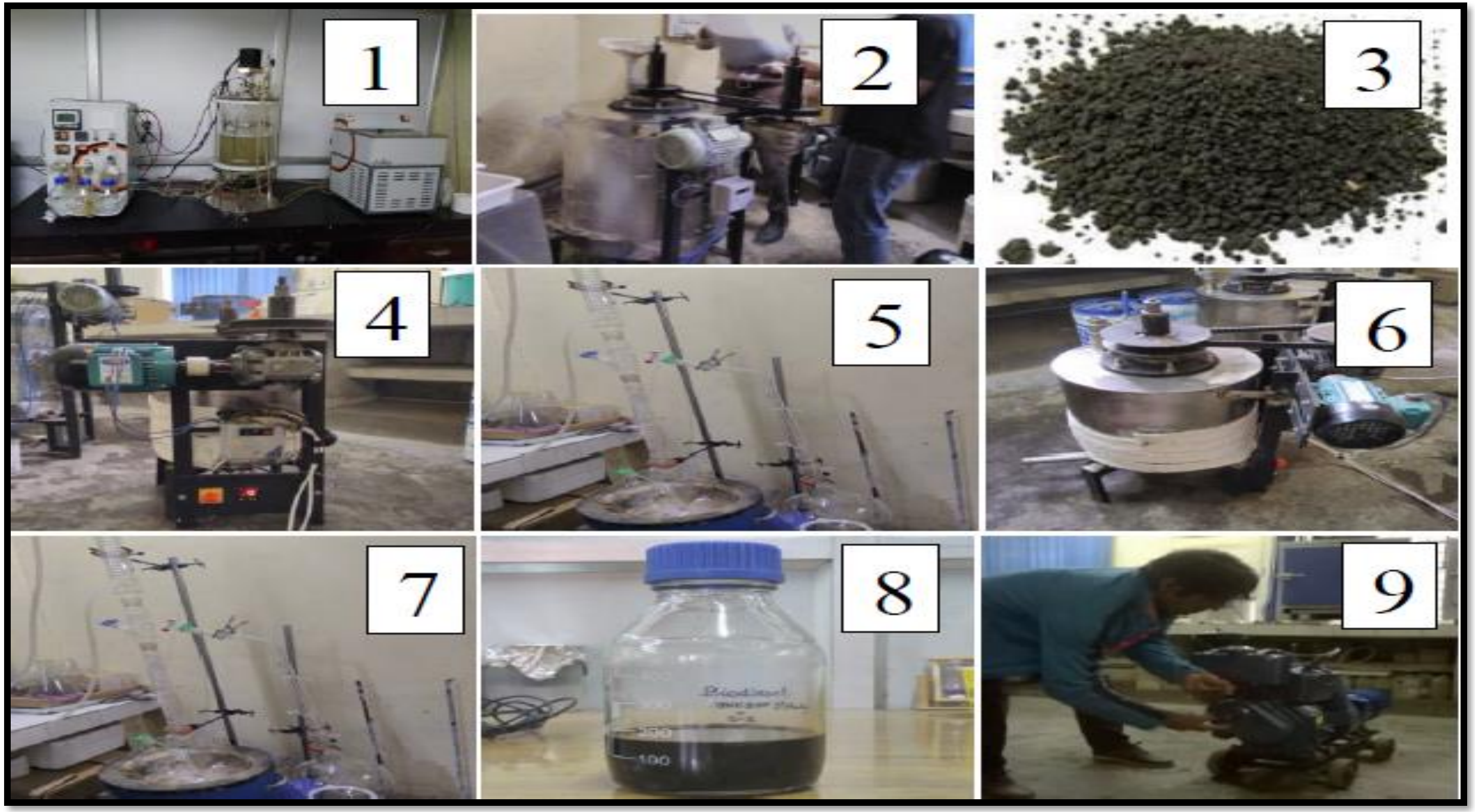
- raw materials
- chemicals
-fuels
- steam
- electricity
- water

- per ton biodiesel
- glycerin
- Other products
EPS
PHA
Biopyrrole

- Comparable lipid content (up to 25% w/w)
- Naturally producing
- Cost free
- Mitigate disposal pressure

- High oil content (up to 95%)
- Rapid growth rate
- Less affected by climate
- Not require arable land

Production of biodiesel from carbon dioxide sequestrating bacteia and municipal sludge



1. Bacteria growing in fermenter, 2. Sewage sludge from JNU STP amended with bacteria in a 200L reactor, 3. Dried and crushed Amended sludge solids 4. Transesterification using Methanol, 5. Recovery of Methanol 6. Hexane purification of biodiesel, 7. Hexane recovery, 8. Biodiesel obtained 9. Generator successfully tested using 1% to 5% Biodiesel blend

Physical parameters of biodiesel and engine testing

- **Properties of biodiesel**

  K. viscosity : 2.13 cSt at 40^{O}C

  Total acidity : 0.30 mg of KOH/gm

  Flash point : 37^{O}C

  Specific gravity : 0.8320 at 15^{O}C

  Cetane number by calculation : 56

  Cloud point: -10 ^{O}C

  Pour point: -18 ^{O}C

  Heating value (GCV) : 45796 KJ/kg.

- **Energy efficiency**

  1 to 5% blended biodiesel were tested in genset (2.5 kW) show the enhanced run time from 11 minutes/100 ml (conventional diesel) to 19 minute/100 ml (5% blended diesel v/v).

- **Emission analysis**

  5% blended diesel emission were analyze by gas analyzer CO_2 16-18%, CO 0.10-0.11%, HC 40-47 ppm, O2 17-18%, which is quite comparable to conventional diesel emission measured by gas analyzer which is CO_2 15-18%, CO 0.09-0.11%, HC 38-45 ppm, O_2 16-18%.

Production of recombinant carbonic anhydrase for synthesis of bio-composite materials

Serratia sp.

Genomic DNA

5' GGATCCATGCTGCGTAAAAACCCCAGTG 3'

5' GAATTCTCAAAACTCATTGCGTAATGCCTGG 3'

Amplification

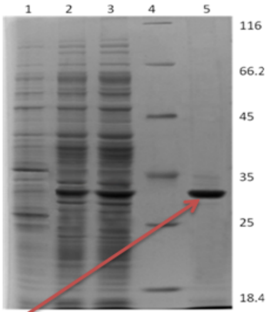
Amplified product ligation pJET vector

Transformation in DH5α

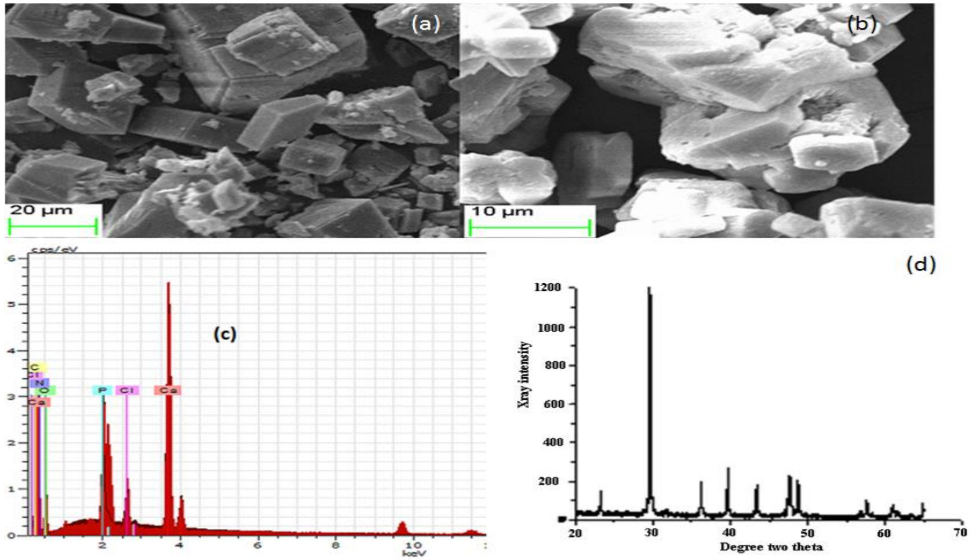
RE digestion

Transform in DH5α

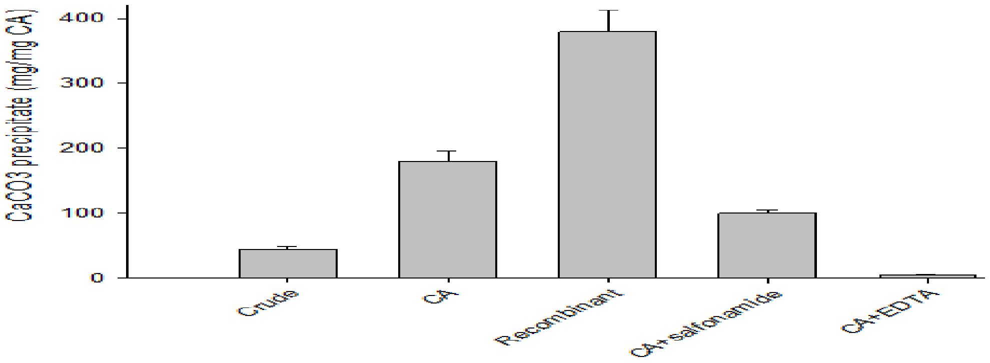
Plasmid transform in BL21 strain of *E.coli*



SDS-PAGE analysis of recombinant CA expressed in *E. coli* Lane 1, soluble fraction of *E. coli* transformed with pET21b(+), Lane 2, soluble fraction of *E. coli* transformed with pET21b(+) containing carbonic anhydrase gene induced with 0.5mM IPTG, Lane 3, soluble fraction of *E. coli* transformed with pET21b(+) containing carbonic anhydrase gene induced with 1 mM IPTG, Lane 4, molecular marker, Lane 5, purified carbonic anhydrase enzyme.



Formation and analysis of calcite for biomaterials



Production of calcium carbonate

Sol-gel methods for calicium silicon af different temperature

Maheshwari, N. et al. 2018. Journal of CO_2 Utilization 27, 423-432.

Production and partial purification of lipase enzyme

Sampling and isolation of bacteria

1. Lipase producing baceria- Psychrophilic bacterial strains isolated from sediment samples from Pangong lake (33°43’04.59”N:78°53’48.48”E), Ladakh, J & K, India.

2. Identification-The isolate identified by 16S rDNA sequencing as *Pseudomonas* sp. ISTPL3.

3. Growth attributes of bacterial strain characterized in different temperature, pH and salinity conditions indicated psychrotolerant, alkalophilic, maximum growth 5% NaCl concentration and tolerant to 50%, v/v Methanol, Ethanol and Propanol.

4. Partial purification-Lipase was purified by precipitation, dialysis, chromatography and Sephadex G-100 gel filtration, molecular weight approx. 31 kDa by SDS-PAGE, resulting in a purification fold of 6.53 and yield of 5.45%.

5. Upon biochemical characterization lipase active in organic polar solvents and sensitive to detergents.

6. Assay method- Lipase was determined spectrophotometrically using p-NPP (Para nitro phenyl palmitate) as asubstrate. One unit of lipase activity was defined as the amount of enzyme which
liberated 1 µmol of p-nitro phenol per min from p-nitro phenyl palmitate.

Preparation of catalytic biocomposite materials by calcite and activated biochar

Pine wood biochar- phosphoric acid and water (1:1 v/v) to soak for 12 h, pyrolyzed at 300 °C for 5 h, washed and dried, and reactivated by KOH (2.0M).

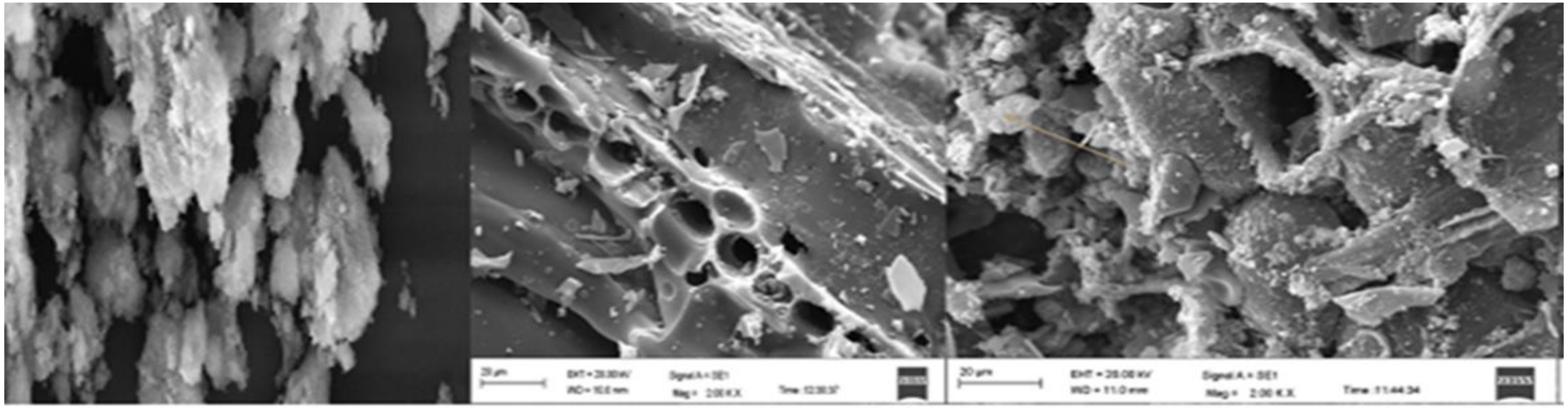
The biochar catalysts prepared by impregnation of activated biochar (7%) with calicite (3%), stirring for 1 h, drying 105 ^{O}C for 24 h, followed by 2 h activation at 600-800 ^{O}C within nitrogen atmosphere.

The glass ceramic bioactive material prepared by sol-gel process (0.2053 g Si powder, 0.192 g $NaNO_3$, 0.6 g calcite) to maintain the molar ratio of SiO_2, Na_2O and CaO respectively similar to crystalline phase $Na_2Ca_2Si_3O_9$, dried and heating from 200-1200 ^{O}C.

Sol-gel methods at different temperature

Enzyme was immobilized on 5 ml syringe filled with 2 g biomaterials, washed, lipase enzyme adsorbed, recirculated. After 24 h, column was washed three-times with 0.1 MPhosphate Buffer (pH 7) and the elute was assayed for lipolytic activity by p-NPP assay and protein content by Bradford method. Immobilization yield (IY) and immobilization efficiency (IE) were calculated.

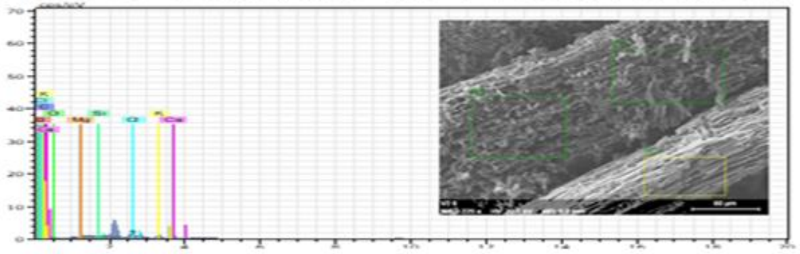
Characterization of catalytic biocomposite materials-I



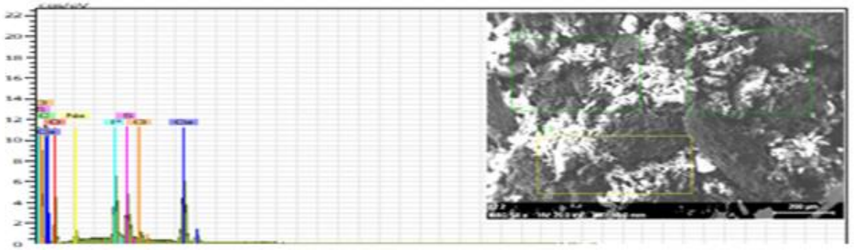
Calcite ppt | Pristine biochar | Biochar + calcite

Pore size of biochar in the range of 4.0-10.0 µm

EDX Analysis of Pristine and calcite immobilized biochar



Element	C norm. [wt.-%]	C Atom. [wt.-%]
Boron	17.07	19.90
Carbon	59.68	62.62
Chlorine	0.90	0.32
Calcium	0.53	0.17
Potassium	0.36	0.12
Magnesium	0.00	0.00
Silicon	0.03	0.02
Oxygen	21.41	16.86



Element	C norm. [wt.-%]	C Atom. [at.-%]
Carbon	38.13	48.34
Calcium	6.24	2.37
Phosphorus	3.84	1.89
Sulfur	2.80	1.33
Sodium	1.06	0.70
Chlorine	0.49	0.21
Oxygen	47.44	45.16

Increase in Ca atomic wt % in calcite immobilized biochar confirmed the immobilization

Characterization of catalytic biocomposite materials-II

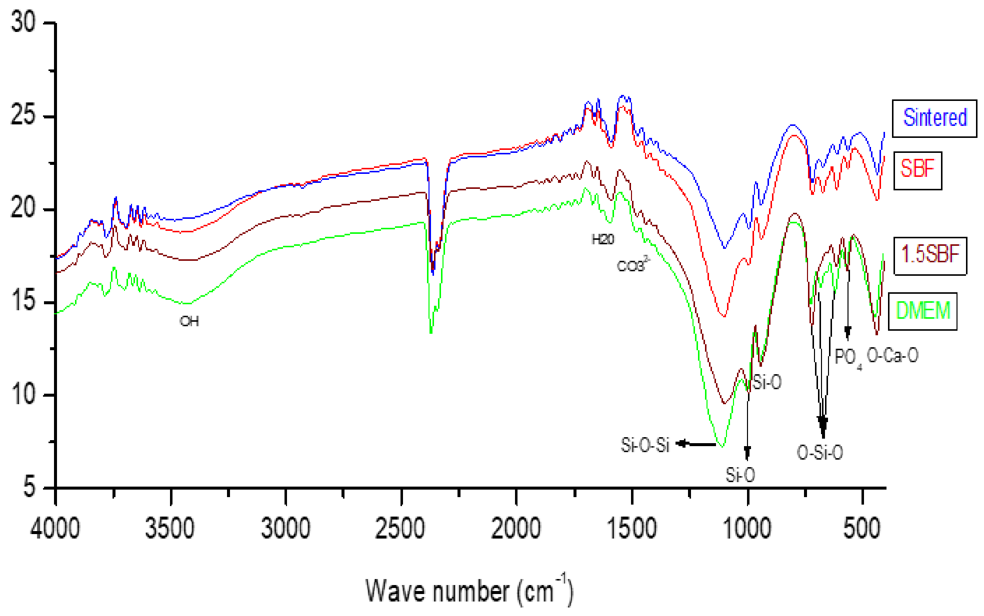
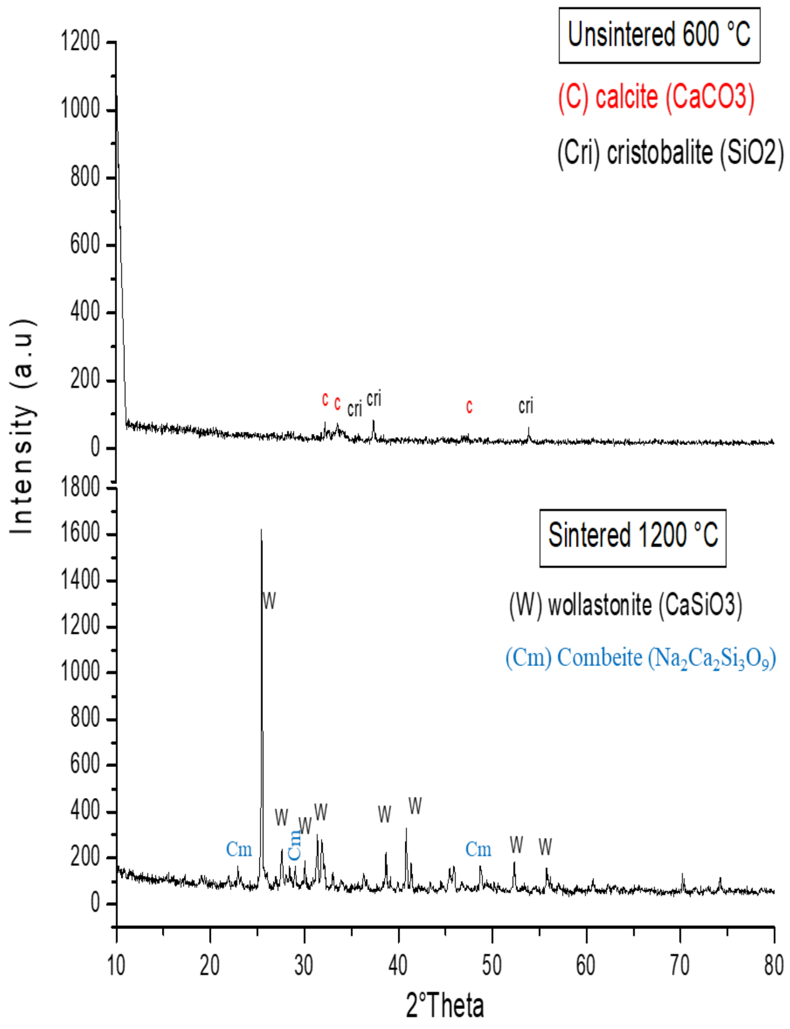


Table.4.2 Representation of peaks and their corresponding annotation material.

Peak(cm^{-1})	Bonds and Corresponding annotation
414.69	O-Ca-O bending
0640.36 and 682.79	O-Si-O bonding
935.47 and 974.04	Si-O stretching modes
1024.19	Symmetrical stretching vibration of Si-O-Si
140.31	Distorted carbonate group
1637.56	Bending vibration of H_2O
3439.07	Moisture absorption band (OH)

FT-IR analysis presence of O-Ca-O, O-Si-O and Si-O-Si functional group..



The XRD analysis indicated important features similar to melt-derived Na_2O-containing glass ceramics formation of crystalline phase $Na_2Ca_2Si_3O_9$

Production of biodiesel by catalytic biocomposite materials

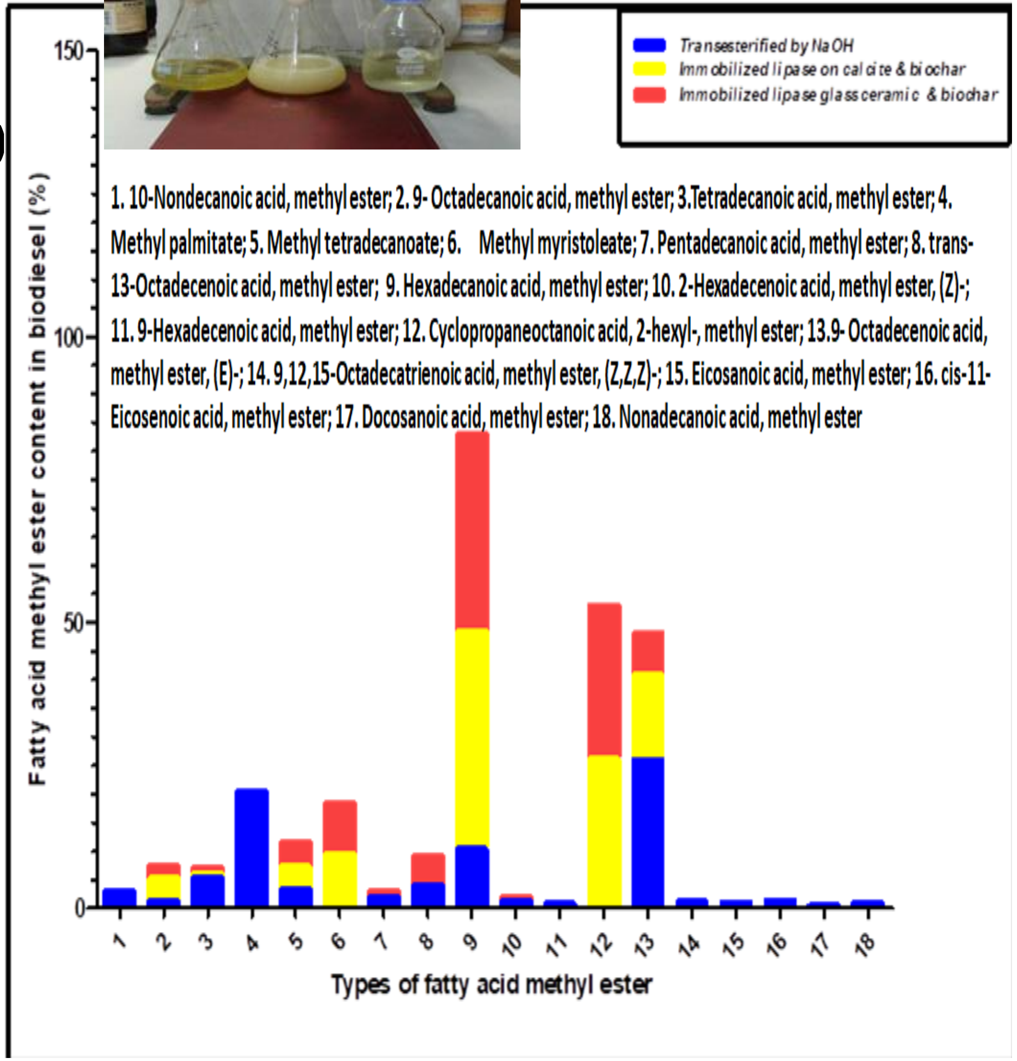
Lipid from *Serratia* sp. ISTD04 extracted by Dyer methods.

Transesterification performed using lipid:methanol molar ratio (1:6) and catalysts-immobilized lipase (100 mg)/ (a) is biochar+lipase, (b) biochar+calcite+lipase, (c) biochar+glass ceramics+lipase and NaOH (1%) and shaken for 3 h at 300 rpm.

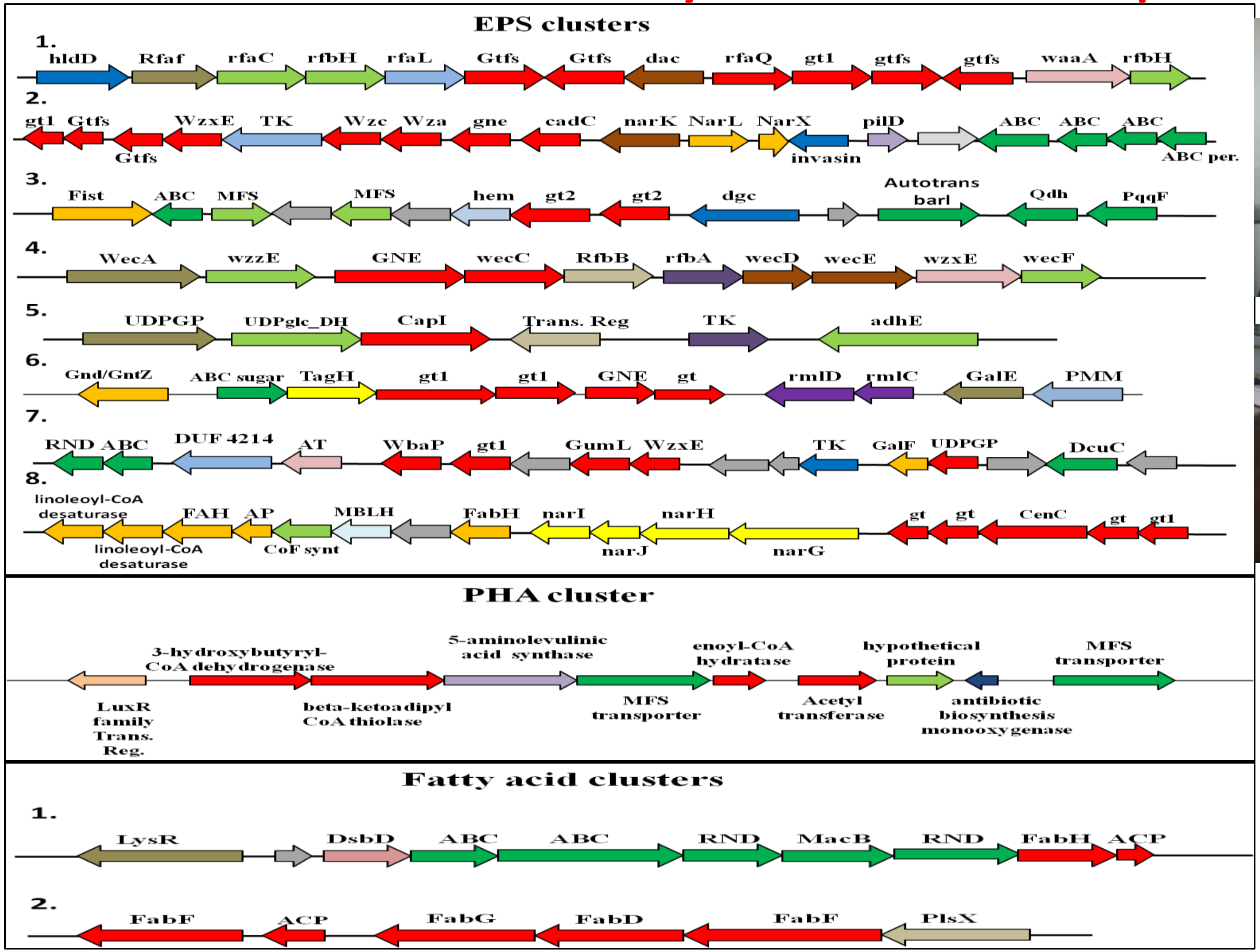
Samples were left overnight for settling of different layers.

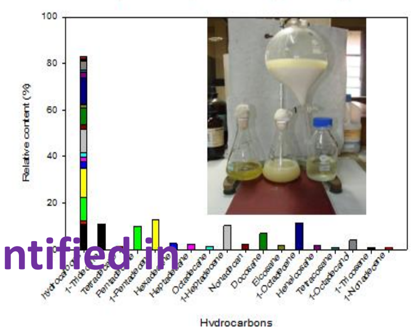
The top layer of biodiesel separated, concentrated, hexane purification subsequent to which the biodiesel content was analyzed through GC/MS.

Reusability of immobilized enzyme indicated immobilization of lipase on calcite enhances more stability and activity and improves recyclability (7 times) makes the use of enzyme as catalysts much more cost effective and viable.



Production of biomaterials by carbon dioxide sequestrating bacteria





Represents putative gene clusters with contig number, position and size identified in *Serratia sp.* ISTD04 genome responsible for polysaccharide production.

Scientific Reports | *(2019) 9:4270 | https://doi.org/10.1038/s41598-019-41052-0*

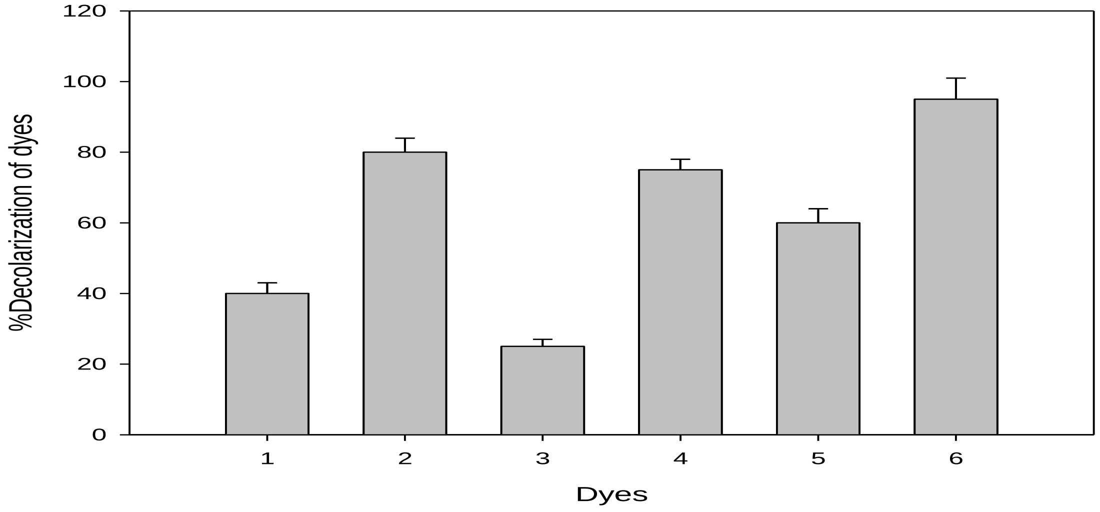
EPS production from *Serratia* sp. ISTD104 and its application in dye decolourization

Production of EPS in 20 litre bioreactor : 8g/litre

Leachate treatment and color removal

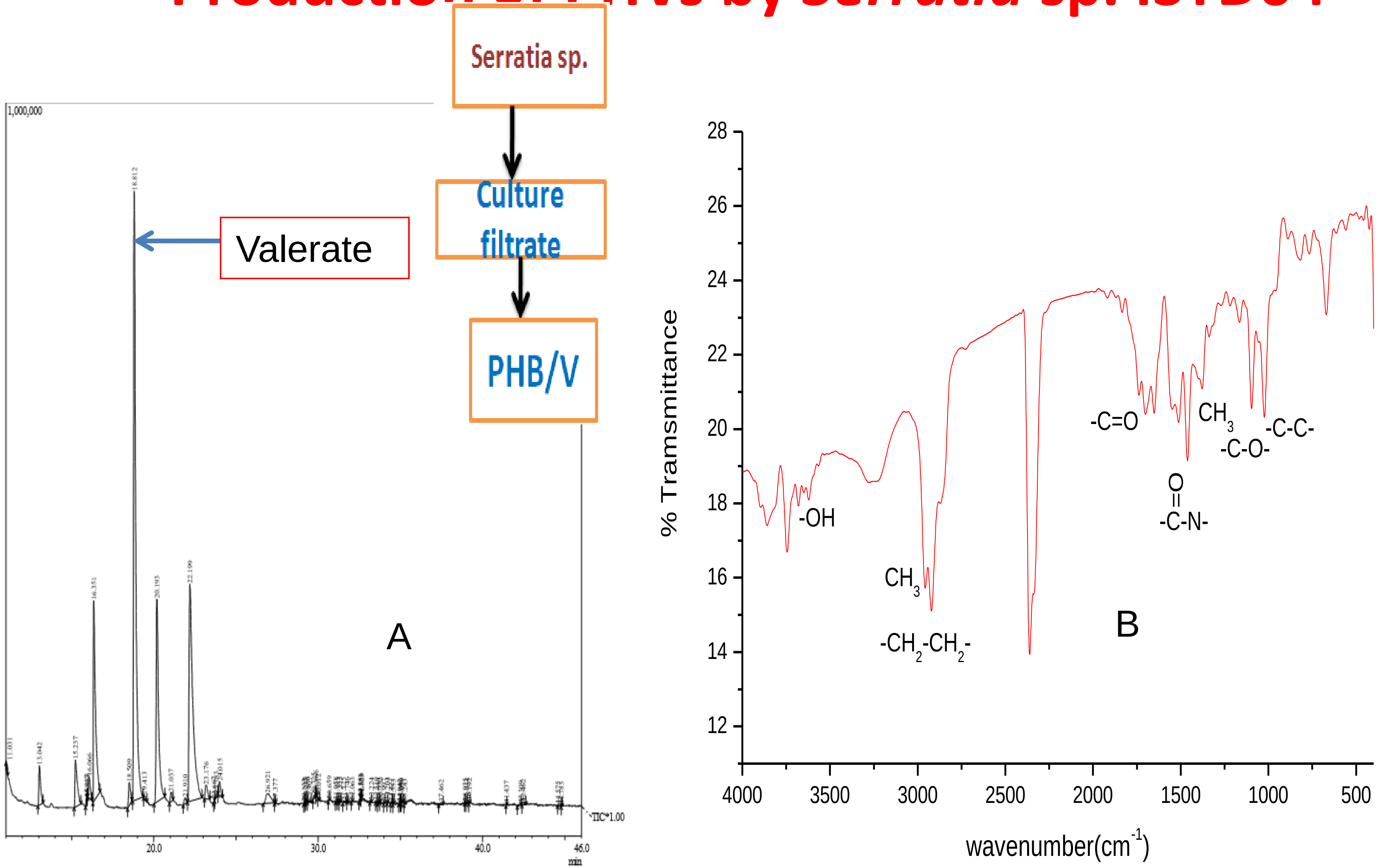
Application of EPS

Kaolin test for flocculating activity



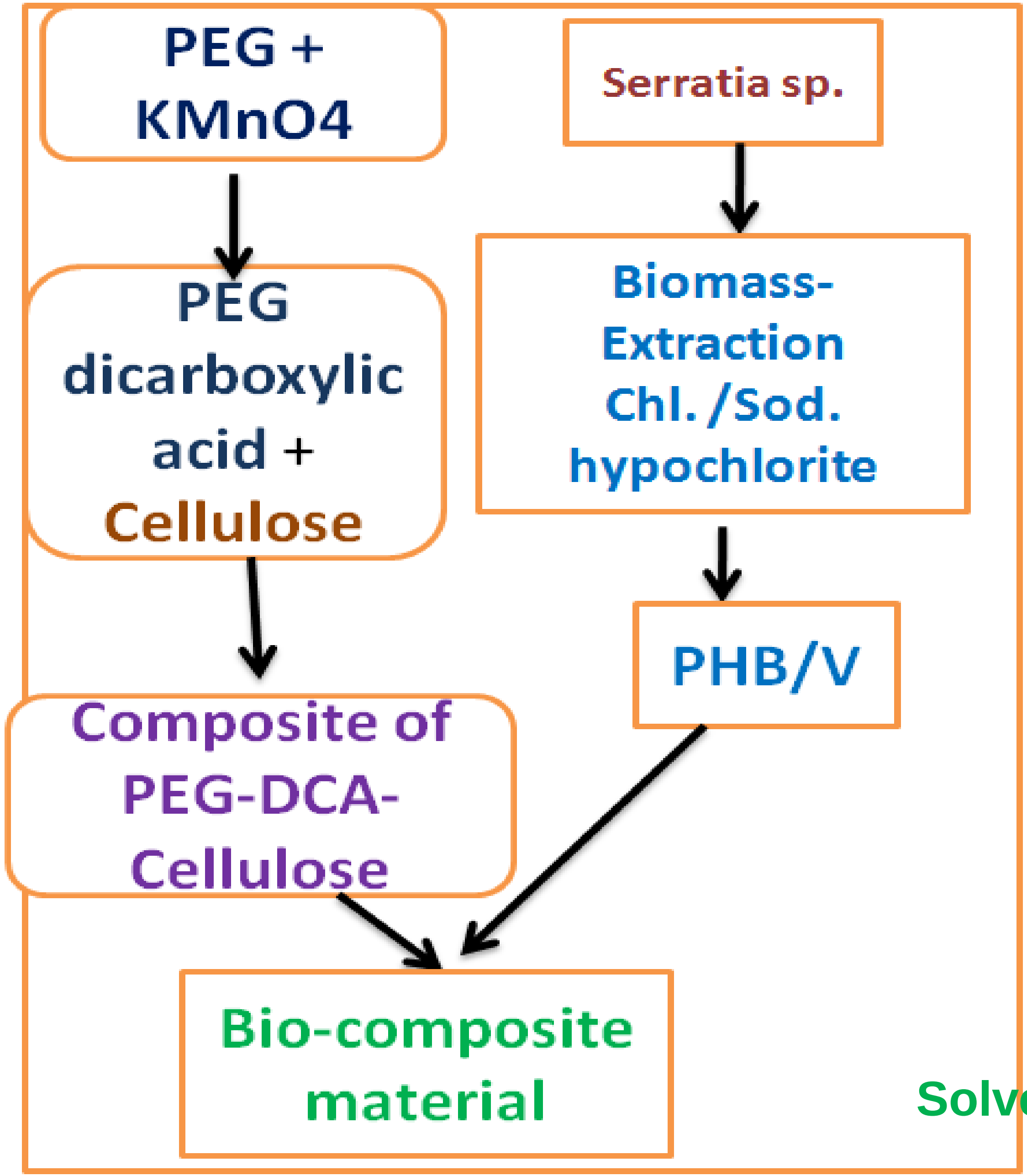
1) Trypan blue (2) Acryl orange (3) Methyl orange (4) Bromothymol blue (5) Aniline blue (6) Crystal violet after 1ml of bacteria culture supernatant Experiments were carried out in triplicate (mean±SD)

Production of PHVs by *Serratia* sp. ISTD04



(A) GC-MS chromatogram of PHAs; (B) FTIR analysis of PHAs

Preparation of bio-composite material from PHV of *Serratia*



Composite of PEG-DCA-Cellulose

Solvent casting of PHV on modified cellulose


Agri-voltaic
At
Amity University Haryana

Objectives

- Create Agri-voltaic plant under conventional ground mounted solar PV plant
- Involvement of Local Community in developing the plant
- Analyze the opportunities and Challenges involved in the process

Site Preparation


Plantation at Solar Farm

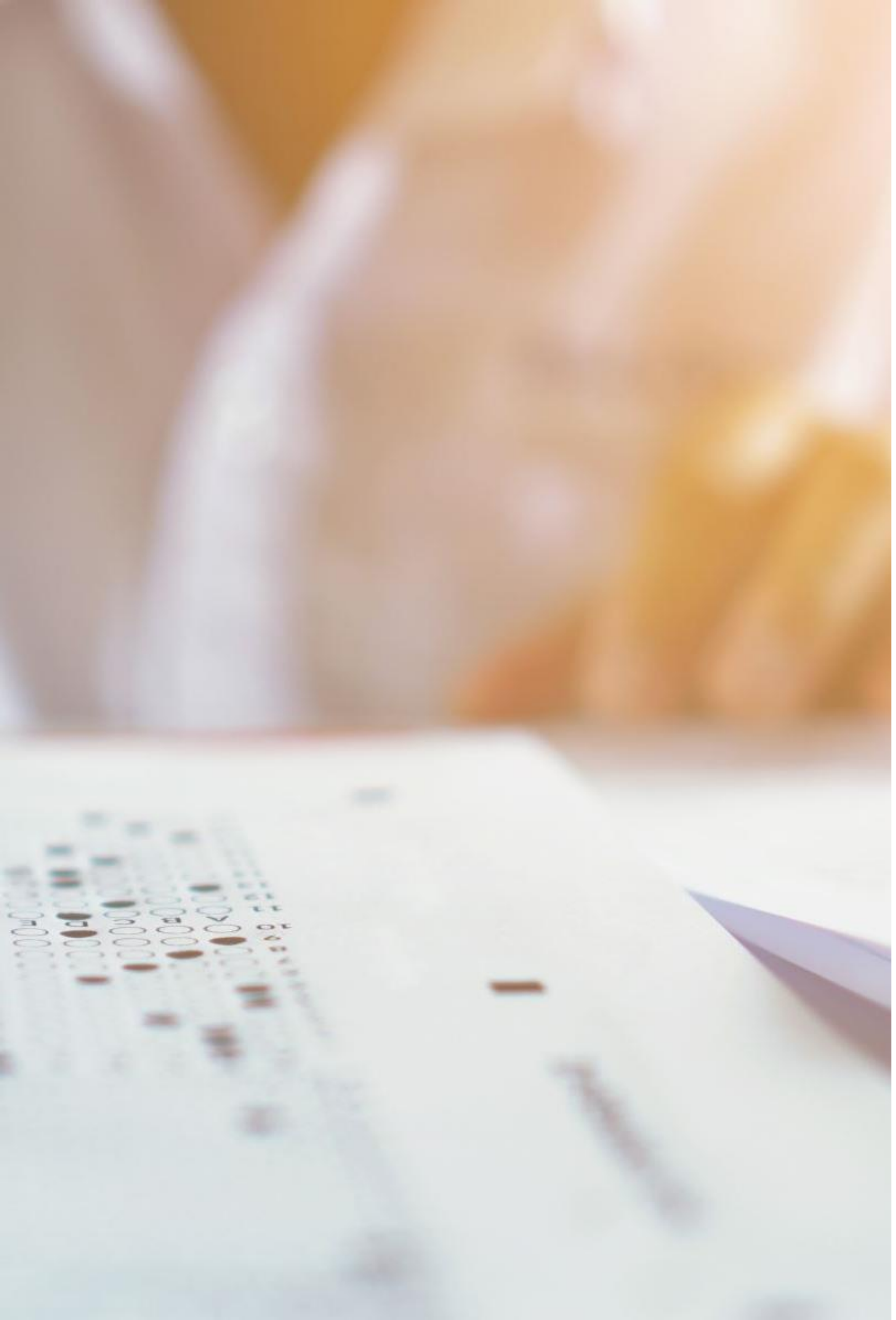
Study to be conducted

- Growth of Plant
- Leaf Index
- Impact on the Chlorophyl
- Diseases of plant
- Weed density
- Soiling of the panels
- Temperature of the panels
- Output from the PV powerplant
- Economics

Flowering & Fruit Bearing in Plants after 1 month

Some of the Findings

- Growth of plant in the sunlight were found to be better than those in the shade.
- Chlorophyll content in plants grown under the shade and under sunlight were found to be same.
- Provide shelter to birds and animals.
- Have threat from Wild animals like peacock, blue bucks



Outcomes

- 1 Major Project conducted by M Sc Renewable Energy final year student.
- 2 Research Paper under preparation to be communicated to Journal.

Research Group

Core Team Members

- Prof Subhra Das, Solar Engg. Dept, AUH
- Dr Viveak Ballyan, HR, AUH
- Ms. Priya Bameta, Horticulture Department, AUH
- Ms. Monika, Horticulture Department, AUH
- Mr Rakesh Dhariwal, Solar Lab Assistant
- Mr Jitender, ME Lab Assistant

Farmers

Mr Mahender Singh
Mr Ishwar
Mr Sandeep

Consulting Faculty from AUH

- Dr Nitai Debnath, AIB, AUH
- Dr Chandershekhar, Physics Dept.
- Dr G K Rao, Physics Dept.

PG Student

Ms. Shreya Bhattacharya, M Sc RE Sem IV

Consulting Faculty outside Amity

Prof. Madhumita Banerjee, Delhi University

Thank You

State of Art Laboratory Infrastructure for Research & Development developed by ACOAST

Amity University Haryana (AUH), Gurugram, has a well-established Institute, Centre of Excellence, ACOAST/ACESH, with a cutting-edge technology-based Air Quality Monitoring System (AQMS) that is being dedicated towards real-time monitoring of Air Quality, which has direct bearing on the ground-reaching solar radiation. We take this opportunity to highlight some of these resources to emphasize on our commitment for testing of solar PV material against environmental conditions, and forecasting of incoming solar radiation through the presence of various atmospheric constituents including aerosols and precursor gases which attenuate the incoming

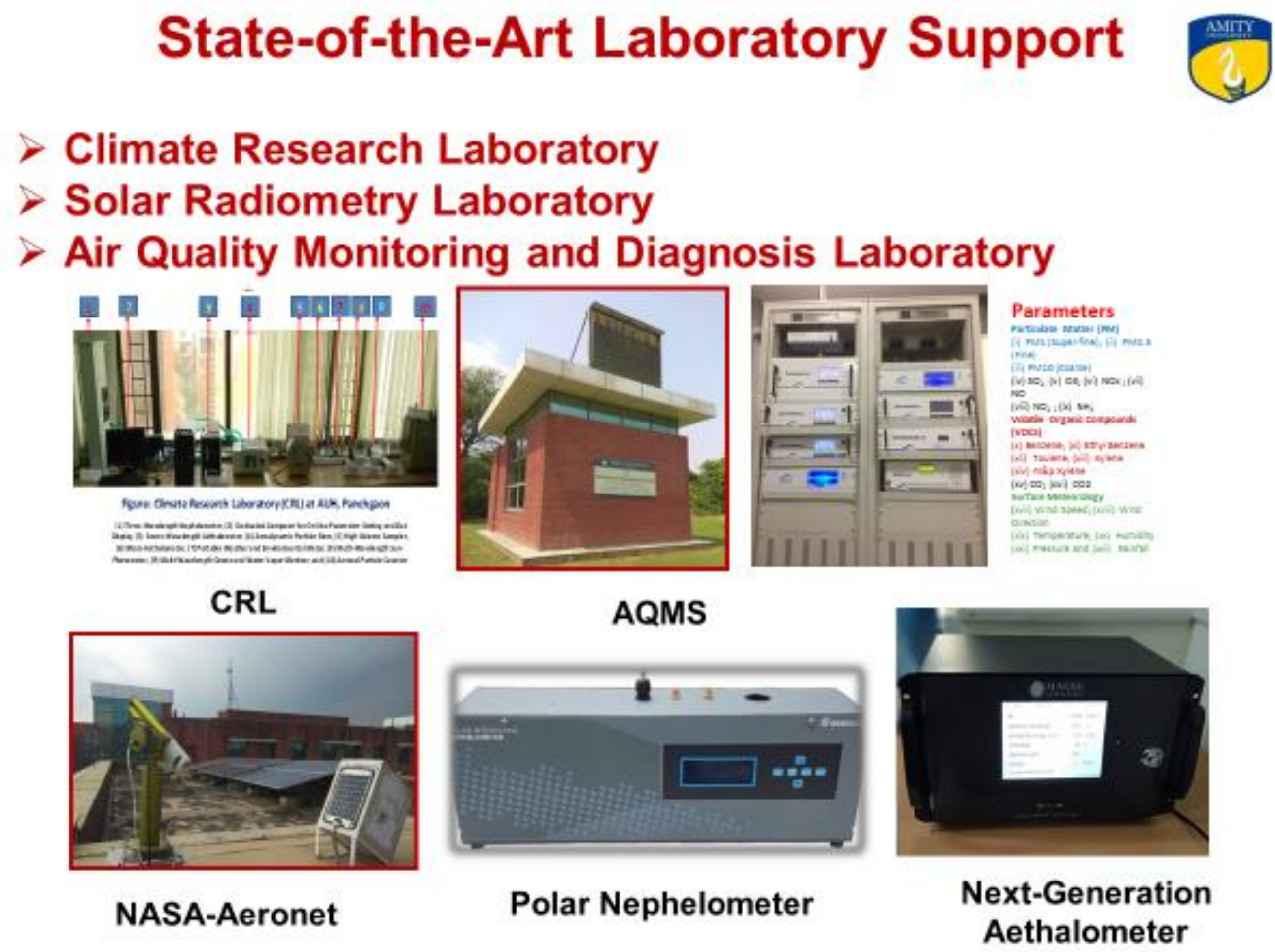


Fig. 5. Experimental Facilities Operating at AUH for Air Quality and Allied Studies.

solar radiation. The Climate Research Laboratory (CRL), including solar radiometers (measuring solar irradiance over a spectral region covering from UV to NIR), is depicted in Figure 3. The Benzene, Toulene, Xylene, MP Xylene, Wind Speed, Wind Direction, Temperature, AQMs is a versatile, real-time system that yields 24x7 high-resolution data composed of 22 parameters (PM_1, $PM_{2.5}$, PM_{10}, CO, CO_2, NOx, NO_2, NO, NH_3, SO_2, O_3, Benzene, Ethyl Humidity, Pressure and Rainfall), describing the quality of air in and around the AUH campus in Panchgaon and its surroundings. Air Quality studies have also been carried out by AUH during dust storms and festive periods (Devara et al., 2017), and significant results related to Particulate Matter (primary aerosols) and Gas Constituents (secondary aerosols) over Panchgaon (a rural station, ~50 km away from Delhi) have been published (Devara et al., 2016; Devara, 2018; Abhijit et al., 2018; Dumka et al., 2019).

The above emission inventory over a rural station, in conjunction with such data from other network stations in the country, serves as a valuable reference (benchmark) input to the models to predict or forecast the incoming solar radiation under different atmospheric conditions such as clear-sky, cloudy-sky, diverse visibility and other turbulent conditions. In AQMS, one can see the Front-view of AQMS with Display Board on the top at AUH, Gurgaon and Calibration Units inside, Rack-mounted PM, Gas Analyzers and Data Portal. Besides the above-mentioned versatile experimental facilities, we also have been using multi-spectral NASA-AERONET sun-sky radiometer, which measure aerosol optical depth that indirectly indicate the solar attenuation in the atmosphere under clear-sky conditions. Very recently, we have also installed a polar nephelometer which measures the linear visibility (directly related to the atmospheric turbidity and hence to ground-reaching solar radiation) and (ii) an Aethalometer which measures black carbon or elemental carbon concentration that contributes absorption of solar radiation and hence warming of the atmosphere.

References

1. Abhijit, C., Devara, P.C.S., Balasubramanian, R. and Daniel A. J. (2019): Aerosol Climate Connection (AC3). Special Issue: An Overview. Aerosol and Air Quality Research, 19, 1-4, ISSN: 1680- 858,doi:10.4209/aaqr.2018.11.0435.
2. Devara, P.C.S., K. Vijayakumar and P. D. Safai (2020): Multi-spectral nephelometer characterization of urban aerosols, Measurement, 154, 107471 (Impact Factor: 3.364).
3. Devara, P.C.S., M.P. Alam, U.C. Dumka, S. Tiwari and A.K. Srivastava (2017): Anomalous Features of Black Carbon Aerosols Observed over a Rural Station during

Diwali Festival of 2015. In "Environmental Pollution, Springer, DOI:10.1007/978-981-981-10-5792- 2_24, pp.293-309.

4. Devara, P.C.S. 2018: Interplay between Climate Change, Air Quality and Health: Measuring, Monitoring and Modelling Techniques, In "Climate Change and Air Quality", Eds. E. Upadhyay and S.L. Kothari, Exel India Publishers, ISBN: 978-93-86724-46-5, January 201
5. Dumka, U.C., Kaskaotis, D.G., Devara, P.C.S., Kumar, R., Tiwari, S., Gerasopoulos, E. and Mihalopoulos, N. 2019: Year-long variability of the fossil fuel and wood burning black carbon components at a rural site in southern Delhi outskirts, Atmospheric Research, 216, 11-25
6. Isha Joon, Subhra Das, Chandra Mohan Srivastava. Fabrication of Metal doped Polymer to study its Thermal & Mechanical Properties. Accepted for publication in Springer lecture notes in Mechanical Engineering 2020. (In Press).
7. Subhra Das. Short Term Forecasting of Solar Radiation and Power Output of 89.6 kWp Solar PV Power Plant. Materials Today: Proceedings, 2020 (in press).
8. Tatenda Kanyowa, Garikayi Victor Nyakujara, Emmanuel Ndala, Subhra Das. Performance Analysis of Scheffler Dish Type Solar Thermal Cooking System Cooking 6000 Meals per Day. Accepted for publication in Journal of Solar Energy

9. S. Das, G. Kwinjo, T. Munetsiwa. Design & Fabrication of steam generator coupled with storage tank using nanofluids as heat transfer fluid. Accepted for 5th Green and Sustainable Chemistry conference, Nov 2020. Paper will be published in special issue of *Current Opinion in Green and Sustainable Chemistry* and Sustainable *Chemistry and Pharmacy.*
10. Heena Yadav, Subhra Das, Sudip Majumder. Synthesis and Characterisation of a nanoalloy for thermal applications. International Conference on Recent Trends in Materials and Devices (ICRTMD), 18th- 19th Dec. Amity University Noida, 2019, ISBN 978-93-86238-82-5, pp 64. Accepted for publication in Springer 's Scopus Indexed International volume, under Book Series - Springer Proceedings in Physics"

11. Subhra Das. Application of Internet of Things in Solar Thermal Power Generation. Accepted for publication for Industrial Applications of IoT and Cloud 2020, Elsevier.
12. Dr S. Das, T Munetsiwa, V. Kundu, T. Kanyowa, E. Ndala, G. Kwinjo and G.V. Nyakujara. Estimation of performance of a solar photovoltaic power plant using

computer simulation. Development of Solar Power Generation and Energy Harvesting, Centre for Sc & Tech of the Non Aligned and other developing countries, Chapter 19, 217-225.

13. Subhra Das**,** Miss. Sarreen Sara Solomon, Mr Avdhesh Saini, "Thermal Analysis of Paraboloid Dish Type Solar Cooker. IOP Journal of Physics conference series, 2018.
14. Sudip Majumder, Neha Sharma, Subhra Das, Namita Pandey, Tapasya Srivastava, Debasree Ghosha. Synthesis, Characterization of Novel PLGA Encapsulated Indole Nanoparticles and Study of its cytotoxic potential against A549 lung cancer cell line. Journal of Applied Pharmaceutical Science, Vol. 8(08), pp 144-150, August, 2018