Rules for Pre-D.Phil. Course Work, Centre of Biotechnology
University of Allahabad, Allahabad

1. Course Work

(a) Every student admitted to the Centre for D.Phil Program will be required to pass the course work

(b) The candidate can submit his /her thesis only if he/she has passed the course work.

(c) The course work will be of 1 semester duration for D. Phil of 24 credits.

(d) The courses are

Paper 1: Basics in Computers and their applications in Biology.
Paper 2: Research Methodology
Paper 3: Electives: Research Project oriented Specific courses

These courses will be prescribed by the Doctoral Program Committee (DPC). The number of courses for each candidate will be decided by the DPC out of the available courses offered by the Center. One elective course has to be selected.

(e) Each discipline will announce the courses to be offered in a given semester prior to beginning of the semester and research scholar will register themselves in consultation with DPC, for different courses.

2. Examination and Evaluation:

a) Only the end semester examination will be held for the D.Phil courses offered in a given semester.

b) Attendance requirement will be as per D.Phil Ordinances.

c) A candidate, who does not fulfill the above requirement, will not be allowed to appear in the concerned examination.

d) Preparation and presentation of seminar on research plan proposal and the other seminar, where applicable, and the review of literature, if applicable, will be evaluated by the DPC while the research theme-specific course will be evaluated by examiner(s) appointed by Program Committee of the Centre.

e) Based on the performance in the examination and other assessment, the candidate will be declared pass or fail. A candidate will be declared pass in both the courses if he/she secures at least 50% marks in each of the courses. This will be in conformity with the recommendations of the special committee appointed by the Vice-Chancellor for common D.Phil rules. For other items like literature review etc., the candidate will be declared passed or failed based on the assessment by the DPC without assigning any marks.

f) The preparation and presentation of research plan/ proposal as required under the D.Phil. ordinance will be evaluated by the DPC as well as supervisor and the candidate will then be declared having passed or failed.

g) The candidate will be considered to have passed the course work of D.Phil program only when he/she has passed all the prerequisites of D.Phil courses.

h) Not with standing above, all recommendations of Special Committee appointed by the Vice-Chancellor for common D.Phil rules will be applicable here.

SYLLABUS FOR THE PRE-D.PHIL. COURSE WORK AT THE CENTRE OF BIOTECHNOLOGY

Specific Objectives: To train the research students in the analytical tools required during the D. Phil. Course and to develop computational skills.

Paper – I: CREDITS= 8

Title of the Paper: Research Methodology

Unit-1 :

a. **Biostatistics**: Measures of Central tendency and Dispersion. Probability distribution: Binomial, Poisson and Normal. Parametric and Nonparametric statistics, Confidence Interval, Errors.
b. **Quantitative Techniques**: Levels of significance, Regression and Correlation, Use of Statistics in Biosciences, Use of Computers in Quantitative analysis.

Unit –2:

Scientific Writing: **An Insight into Research**:

a. Definition and basic concepts, objectives, significance and techniques of research, finding research materials – literature survey, compiling records.
b. Definition and kinds of scientific documents – research paper, review paper, book reviews, thesis, conference and project reports (for the scientific community and for funding agencies).
c. Components of a research paper– the IMRAD system, title, authors and addresses, abstract, acknowledgements, references, tables and illustrations.
d. Dealing with publishers – submission of manuscript, ordering reprints.
e. Oral and poster presentation of research papers in conferences/symposia.
f. Preparation and submission of research project proposals to funding agencies
g. IPR

Unit 3:

Techniques in Molecular Biology:

a. Identification and characterization of DNA, RNA, plasmids.
b. Agarose gel electrophoresis, ethidium bromide staining.
c. Southern, Northern, Western Blotting,
d. RAPD, RFLP, DGGE, TGGE, PCR.

Unit- 4:

Research Techniques:

a. Enzyme assay, enzyme activity and specific activity determination. Cell disintegration and extraction techniques, separation of proteins by fractionation (ammonium sulphate, organic solvents).
b. Ion exchange chromatography, molecular sieve chromatography, affinity chromatography, paper chromatography, thin layer chromatography, ultra filtration, Ultracentrifugation. Gel electrophoresis, isoelectric focusing and immunoelectrophoresis, capillary electrophoresis, pulse field electrophoresis.
c. Microscopy,
d. HPLC, HPTLC, GC-MS, FTIR, SEM/TEM, NMR, AAS.

Suggested reading:

1. Biostatistics: A foundation for Analysis in the Health Sciences 7/E Wayne W. Daniel, Wiley Series in Probability and Statistics.
2. Introductory Statistics. Fifth Edition. (2004) Prem S. Mann. John Wiley and Sons (ASIA) Pvt. Ltd.
3. Bioinformatics Methods and Applications Genomics, Proteomics, and Drug Discovery (S. C. Rastogi, N. Mendiratta, and P. Rastogi).
4. Introduction to Bioinformatics, (Atwood, T. K. and Parry-Smith, D. J).
5. Protein Purification by Robert Scopes, Springer Verlag Publication, 1982
6. Tools in Biochemistry, David Cooper
7. Methods of Protein and Nucleic acid Research, Osterman Vol I – III
8. Centrifugation D. Rickwood
9. Practical Biochemistry, V th edition, Keith Wilson and Walker.
10. Bioinformatics by David Mound
11. Practical Biochemistry, Vth edition, Keith Wilson and Walker.

Paper 2: CREDIT= 6

Basics in Computers and their applications in Biology.

Unit- 1

Basic Concepts of Computer:

a. History of Computer,
b. Concept of Computer hardware,
c. Concept of Computer languages
d. Concept of Computer Softwares

Unit-2

Computer applications in Biology:

a. Spreadsheet tools : Introduction to spreadsheet applications, features, Using formulas and functions, Data storing,
b. Features for Statistical data analysis, Generating charts / graph and other features, Tools – Microsoft Excel or similar.
c. Presentation tools : Introduction, features and functions, Presentation of Power Point
d. Presentation, Customizing presentation, Showing presentation, Tools – Microsoft Power Point or Similar.
e. Web Search: Introduction to Internet, Use of Internet and WWW, Use of search engines,
f. Biological data bases.

Suggested reading:

1. Genetic Programming: An Introduction on the Automatic Evolution of Computer Programs and Its Application, Wolfgang Banzhaf

2. Computational Biology: Unix/Linux, Data Processing and Programming By: Robbe Wunschiers Edition: 1st edition, November 2004 Format: Paperback, 284pp Publisher: Springer-Verlag New York, LLC Edition: 1st edition, July 2002 By: Christopher Fall (Ed), Eric Marland (Ed), John Wagner (Ed), John Tyson (Ed) Format: Hardcover, 448pp, Publisher: Springer Verlag, ISBN: 0387953698

3. Computational Modeling of Genetic and Biochemical Networks
   By: James M. Bower (Ed), Hamid Bolouri (Ed), Edition: 1st edition, January 2001, Format: Hardcover, 390pp, Publisher: MIT Press, ISBN: 0262024810

4. Computational Analysis of Biochemical Systems: A Practical Guide for Biochemists and Molecular Biologists By: Eberhard O. Voit, Edition: Book and CD-ROM edition, September2000, Format: Paperback, 544pp, Publisher: Cambridge University Press, ISBN: 0521785790

5. Computational Molecular Biology: An Introduction By: Peter Clote, Rolf Backofen, Edition: 1st edition, September 2000, Format: Paperback textbook, 300pp, Publisher: John Wiley & Sons, ISBN: 0471872520

6. Introduction to Computational Biology: Maps, Edition: 1st edition, June 1995, Format: Hardcover textbook, 431pp, Publisher: CRC Press, ISBN: 0412993910

7. Sequences and Genomes By: Michael S. Waterman, Introduction to Computational Biology, Maps, sequences and genomes, Michael S. Waterman, Chapman & Hall--CRC Press ISBN 0 412 99391 0

Paper – 3 (Elective) **CREDIT=10**

Select any one paper related to the research work of the proposed supervisor. The course will be in the form of theory examination (5 credits) and presentation of review of published papers in the specific field (5 credits).

BT-1 :

Paper: Biotechnological application of hazardous waste management and management of resources.

Unit 1:

a. Use of microbial systems.

Unit 2:

a. Phytoremediation.
b. Waste water treatment using aquatic plants,
c. Root zone treatment.

Unit 3:

a. Bioremediation, *in situ* and *ex situ* bioremediation
b. Constrains and priorities of bioremediation
c. Evaluating Bioremediation
d. Bioremediation of VOCs.

Unit 4:

a. Need for management of resources.
b. Role of environmental biotechnology in management of resources.
c. Reclamation of wasteland, biomass production,
d. Biogas and biofuel production.
e. Development of environmentally friendly processes such as integrated waste management.

Unit 5:

a. Sources of heavy metal pollution
b. Microbial interactions with inorganic pollutants
c. Microbial metal resistance
d. Microbial transformation, accumulation and concentration of metals
e. Biosorption Biotechnology and heavy metal pollution.

Suggested reading:

1. Biotreatment Systems , Volume II ; D.L. Wise.
2. Advances in Biotechnological Process ; Mizrahi & Wezel.
3. Bruce Rittman, Perry L. McCarty. Environmental Biotechnology: Principles and Applications. McGraw-Hill 2nd edition (July 25, 2000) ISBN: 0072345535.
4. Raina M. Maier , Ian L. Pepper, Charles P. Gerba. Environmental Microbiology. Publisher: Academic Press; (February 23, 2000).
5. Martin Alexander. Biodegradation and Bioremediation. Academic Press; 2nd edition (April 15, 1999) ISBN: 0120498618.

6. Gabriel Bitton (Author). Wastewater Microbiology, 2nd Edition. Wiley-Liss; 2nd edition (February 16, 1999) ISBN: 0471320471.
7. Milton Wainwright. An Introduction to Environmental Biotechnology. Kluwer Academic Publishers, Boston. Hardbound, ISBN 0-7923-8569-1.July 1999, 192.
8. M.N.V. Prasad, Kazimierz Strzalka. Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer Academic Publishers, Dordrecht Hardbound, ISBN 1-4020-0468-0. February 2002, 460 pp.
9. Biotechnology for Solving Agricultural Problems; Danforth & Bakst.
10. Introduction to Environmental Microbiology; R. Mitchell.

BT 2: Paper- Advanced Techniques in Cell Culture

Unit 1:

Basic techniques in tissue culture:

a. Introduction to Cell & Tissue Culture.
b. Design & lab setup of Tissue Culture laboratory,
c. Tissue culture Media (Composition preparation),
d. Types of culture.
e. Role of Plant Hormones in growth & development of Plants.
f. Micro propagation (Organogenesis, Somatic Embryogenesis, Shoot tip culture, Rapid clonal propagation, Embryo Culture & Embryo Rescue, Acclimatization of Plants)
g. *In vitro* mutagenesis. Cryopreservation, Slow growth & DNA Banking for germplasm conservation.

Unit 2:

Plant cell culture, plant transformation technology & its applications:

a. Basics of Tumor formation, Hairy root
b. Mechanism of DNA transfer, role of Virulence gene
c. Features of Ti & Ri Plasmid , Use of Ti & Ri as vectors, Binary vectors
d. Use of 35s & other promoters, genetic markers methods of nuclear transformation, viral vectors & their applications,
e. Multiple gene transfers: vector less or direct DNA transfer
f. Use of reporter gene
g. Particle bombardment , electroporation, Microinjection
h. Transformation of monocots
i. Transgene stability & gene silencing in Plant transformation
j. Applications of Plant Transformation for Productivity & performance Herbicide resistance like atrazine
k. Insect resistance Bt gene ,non Bt like protease inhibiters, Virus resistance, disease resistance, antibiotic stress, post harvest losses, long shelf life of fruits & flowers
l. Chloroplast transformation
m. Metabolic engineering & Industrial products

Unit 3:

a. Plant secondary metabolites, control mechanisms & manipulation of Phenyl Propanol pathway, Shikimate pathway, alkaloids, Industrial enzymes,
b. Biodegradable plastics, therapeutic proteins, lysozomal enzymes, antibodies, edible vaccines, purification strategies, oleosin partitioning technology
c. Integration of Genetic Engineering of Plants in Agriculture
d. Diseases resistance, Biotic & Abiotic stress

Suggested Reading:

1. An introduction to Plant Tissue Culture 2nd edn. Razdan, M. K, Science Publishers, USA.
2. Textbook of plant biotechnology, Chawla P.K.2002,Oxford&IBH,New Delhi.
3. Bhojwani, S. S. and M. K. Razdan 1996.Plant Tissue Culture: Theory and Practice, Elsevier Pub.
4. Chrispeels, M. J. 2002.Plant Tissue Culture:Genetical Aspects. Jones and Bortlett Publishers, International.
5. Chopra V. L. et al 1999. Applied Plant biotechnology. Science Publishers Inc.
6. Verpoorte, R. and A.W. Alfermann (Eds) 2000.Metabolic Engineering of plant secondary metabolism, lower Academic Publisher.
7. Kuchler, R.J., Biochemical Methods in cell culture and Virology, Dowden, Huchinson and Ross,Inc. Strausberg, USA, 1977
8. Morgan, S. I. Animal cell culture, 1993,Bio Scientific Publishers Ltd, Oxford.
9. Freshney,R.I.Culture of Animal cells:A Manual of Basic Technique, 1994, John Wiley and Sons Inc. Publication, USA.
10. Butler, M.Mammalian, cell Biotechnology: A Practical Approach (1991), IRL Press, Oxford.
11. Jenni P.Mather and David Barnes, eds; Animal cell culture Methods, Methods in cell Biology, vol.57, Academic Press.
12. Cell Culture: Methods in enzymology,Vol-58,Academic Press 1979 or recent.

BT- 3

Paper: Agricultural Microbiology and Microbial Ecology

Unit 1

a. Soil Enzymes – origin and range of enzymes in soil, methods of measurement and extraction of soil enzymes, interactions between agrochemical and soil enzymes.
b. Recent advances in biological Nitrogen fixation
c. Microbial Biofertilisers.

Unit 2

a. Epidemiology of plant diseases
b. Biological control of soil borne plant pathogens
c. New Directions and Importance of Microbial Ecology

Unit 3

a. Microbiology of the Extreme Environment
b. Hot springs, acid springs and lakes
c. Microbial life in hyper saline environments – ecophysiological aspects, sea and salt lakes.
d. Microbial life at low temperatures
e. Microbiology to 10,500 metres under the deep sea.

Unit 4

a. Anaerobic Microorganisms – ecophysiological aspects, principles and techniques for the isolation, enumeration and identification of Methanogens,
b. Dissimilatory Sulphate reducing and Anoxygenic Phototrophic bacteria
c. Geomicrobiological processes – physiological and biochemical aspects, Methods in Geo-microbiology.
d. Biodiversity as a source of innovation in Biotechnology

Suggested Reading:

1. Soil Enzymes by R. G. Burns
2. Chemistry and Biochemistry of Biological Nitrogen Fixation by J. R. Postgate. Plenum Press
3. Biofertilizers by L. L. Somani and others. Scientific Publishers, Jodhpur
4. Plant Pathology by J. C. Walker
5. Plant Diseases by R. S. Singh
6. Microbial Ecology by J. M. Lynch
7. Bergey's Manual of Systematic Bacteriology Volume 1. Springer
8. Annual Reviews in Microbiology Volumes 46 & 49 by L. N. Ornston, A. Balows and E. P. Greenberg (eds). Academic Press
9. Microbes in Extreme Environments by D. J. Kushner
10. Anaerobes by Shapton. Academic Press
11. Microbiological Aspects of Anaerobic Digestion – A Laboratory Manual by D. R. Ranade and S. V. Gadre (eds) Agharkar Research Institute, Pune.
12. Geomicrobiology by H. L. Ehrlich

BT-4: Paper: Physiology of Stress in Plants

Unit 1:

Types of Stress- Abiotic (Including pollutants) and Biotic.

Unit 2:

Alterations in physiology of plants due to the different stress and their mechanisms to combat them.

Unit 3:

Stress due to prolonged sun light & UV, mechanism of resistance to UV eg. through induction of pigment synthesis, plant repair enzymes

Unit 4:

Stress due to chilling, mechanism of resistance

Unit 5:

Stress due to high temperature, mechanism of avoidance and reduction

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Unit 6:

Stress due to water – Plants and water, chemical & water potential gradients, transpiration, stomatal apparatus, mechanism of opening & closing, antitranspirants, Effect of water stress on accumulation of proline and betaines and their possible role in osmotic adjustment under such conditions.
Drought tolerance/resistance mechanism, Screening methods for water stress tolerant varieties.
Availability of soil water & determination of soil water potential, Mechanism of plant resistance to water logging/ hypoxia.

Unit 7:

Stress due to salinity, mechanism of salt tolerance in higher plants

Unit 8:

Mechanism of plant resistance to nutrient deficiency stress

Unit 9:

Elementary idea of mechanism of plant resistance to heavy metal toxicity
Elementary idea of mechanism of resistance in plants against viruses, fungal pathogen and insects (including Bt technology).

Suggested reading:

1. Basra, A.S. & Basra, R.K. 1997. Mechanisms of environmental stress resistance in plants, Harwood Academic, Publishers, The Netherlands.
2. Chopra, V.L. & Paroda, R.S. 1988. Approaches for incorporating drought and salinity resistance in crop plants, Oxford & IBH Publishing Co. Pvt. Ltd., ND
3. Gupta, U.S. 1985. Physiological aspects of dryland farming, Oxford & IBH Journal of Bioscience, Special issue: Cellular Stress Response, 1998.23(4):Oct., The Indian Academy of Sciences, Bangalore
4. Kramer, P.J. 1983. Water relations of plants, Academic Press Inc., NY
5. Nilsen, L. & Orcutt, 1998. Physiology of plants under stress : Abiotic factor
6. Paleg, L.G. & Aspinall, D. 1981. Physiology and biochemistry of drought resistance in plants, Academic Press, NY.
7. Singh, Randhir & Sawhney, S.K. 1988. Advances in frontier areas of plant biochemistry, Prentice-Hall of India Pvt. Ltd., New Delhi
8. Smallwood, M.F., Calbert, C.M. and Bowles, D.J. 1999. Plant responses to environmental stress, BIOS Scientific Publishers Ltd., USA
9. Taiz, & Zeiger, 1998. Plant Physiology, Sinauer Associates
10. Treshow, M. 1970. Environment and plant response, Mc Graw Hill, N.

BT-5: <u>Paper: Nanobiotechnology</u>

Unit 1:

a. Concept of nanobiotechnology and bionanofabrication, method of characterization of nanoparticles, biological effects and applications.

Unit 2:

a. Biosynthesis of metal and alloy nanoparticles using microbes through chemical route.

Unit 3:

a. Interaction of metal nanoparticles with microbes.

Unit 4:

a. Bacterial spore engineering and applications in nanobiotechnology
b. Delivery vehicle
c. Source of novel self-assembly proteins as biosensor.

Unit 5:

a. Biopolyester (Polyhydroxyalkanoates) nanoparticles, production by microbes and their applications.

Unit 6:

a. Cultivation of magnetotactic bacteria (MTBs),
b. Biotechnological production and applications of magnetosomes.

Unit 7:

a. Alginate biosynthesis and modification by bacteria,
b. Alginate-based bionanostructures.

Unit 8:

a. Applications of bacteriophages
b. rDNA technology, phage display, protein evolution by phage display, bacteriophages as templates for inorganic nanostructures,
c. nanowires and nanorings.

Unit 9:

a. Molecular biomimetics
b. Combinatorial biology approach in selecting inorganic specific peptides
c. Post-selection engineering of inorganic binding peptides.

Unit 10:

a. Bacterial protein complexes: cellulosome and designer cellulosome, streptavidin-biotin.
b. Bacterial protein complexes, S-layer proteins: potential applications in nanotechnology.
c. Ethical, legal, social and environmental issues concerning nanoscience including possible health risks.

Suggested reading:

1. Multilayer Thin Films, Editor(s): Gero Decher, Joseph B. Schlenoff Publisher: Wiley-VCH Verlag GmbH & Co. KGaA ISBN: 3527304401
2. Bionanotechnology: Lessons from Nature Author: David S. Goodsell Publisher: Wiley-Liss ISBN: 047141719X
3. Biomedical Nanotechnology Editor: Neelina H. Malsch Publisher: CRC Press ISBN: 0-8247-2579-4.

BT-6: Paper: PROTEOMICS

Unit 1:
Protein analysis: concentration determination, amino acid composition N-terminal sequencing, limited proteolysis, Posttranslational modifications like acetylation, glycosylation, hydroxylation, oxidation, sulfatation.

Unit 2:
Electrophoresis: Basic principles; 1D and 2-D electrophoresis of proteins; Isoelectric focusing; Capillary electrophoresis.

Unit 3:
Mass spectrometry: Principles, Electrospray ionization, Matrix assisted laser desorption ionization, Tandem mass spectrometry (MS-MS), H-D exchange coupled mass spectrometry, Peptide mass fingerprinting, Peptide sequencing by tandem mass spectrometry, Protein identification with MS/MS data.

Unit 4:
Protein Microarrays: Proteomics Microchips; Nanospray LC/MS; Microarray MS chip (Ion trap); SELDI.

Unit 5:
Comparative Proteomics: Differential display proteomics; Differential Gel electrophoresis.

Unit 6:
Quantitative proteomics: SILAC, iTRAQ and label free analysis.

Unit 7:
Bioinformatics tools to proteomics.

Unit 8:
Structural Proteomics.

Suggested reading:

1. Biochemistry by V. Voet and J.G. Voet (4^{th} edition Dec 2010).
2. Introduction to Proteomics: Tools for the New Biology by Daniel C. Liebler, Humana Press, Totowa, NJ, 1st edition (December 2001)
3. An Introduction to Biological Mass Spectrometry", C. Dass, Wiley, USA, 2002.
4. The Expanding Role of Mass Spectrometry in Biotechnology", G. Siuzdak, MCC Press, San Diego, 2004.
5. Recent reviews and research articles from the journals.

BT-7 Paper: Biological Hydrogen Production

Unit 1:

Introduction to biological H_2 production and applications, organisms involved in H_2 production, biological routes and its commercialization.

Unit 2:

Direct and indirect photolysis of water, cyanobacterial H_2 production, role of enzymes (nitrogenase and hydrogenase), factors affecting cyanobacterial H_2 production, approaches for enhancement, improvement in process by combined cultures, reverse micellar entrapment and genetic engineering.

Unit 3:

Dark fermentative H_2 production, organisms involved in the process, different physic-chemical factors affecting H_2 production, genes and enzymes involved in the process, approaches to enhance H2 production, metabolic engineering.

Unit 4:

Photofermentative H_2 production, organisms involved in the process, metabolic pathways and enzymes involved in the process, genetic studies and approaches for H_2 production enhancements.

Unit 5:

Bioprocess engineering of biological H_2 production, Bioreactor design, Scale-up process, Energy calculations.

Suggested Books/Reviews:

1. Wen-Hsing Chen, Iowa State University, Biological Hydrogen Production by Anaerobic Fermentation. Publisher: Iowa University, 2006, ISBN: 0542996320, 978054299630
2. Das, D. & Veziroglu, TN. 2008 Advances in biological hydrogen production processes. *International Journal of Hydrogen Energy* **33**, 6046-6057.
3. Hallenbeck, P.C. & Benemann, J.R. 2002 Biological hydrogen production; fundamentals & limiting processes. *International Journal of Hydrogen Energy* **27**, 1185–1193.

4. Kars, G., Gunduz, U., Yucel, M., Turker, L. & Eroglu, I. 2006 Hydrogen production and transcriptional analysis of *nif*D, *nif*K and *hup*S genes in *Rhodobacter sphaeroides* O.U.001 grown in media with different concentrations of molybdenum and iron. *International Journal of Hydrogen Energy* **31**, 1536-1544. Kargi, F. & Ozmihci, S. 2010 Effects of dark/light bacteria ratio on bio-hydrogen production by combined fed-batch fermentation of ground wheat starch. *Biomass and Bioenergy* **34**, 869-874.
5. Pandey, A. & Pandey, A. 2008 Reverse micelles as suitable microreactor for increased biohydrogen production. *International Journal of Hydrogen Energy* **33**, 273-278.
6. Pandey, A., Sinha, P., Kotay, S.M. & Das, D. 2009 Isolation & evaluation of a high H_2-producing lab isolate from cow dung. *International Journal of Hydrogen Energy* **34**, 7483-7488.
7. Pandey, A., Srivastava, N. & Sinha, P. 2012 Optimization of hydrogen production by *Rhodobacter sphaeroides*-NMBL-01. *Biomass and Bioenergy* **37**, 251-256.
8. Pandey A. & Pandey A. 2012 Cyanobacterial hydrogen production-A step towards clean environment. *International Journal of Hydrogen Energy* **37**, 139-150.