FINAL SCIENTIFIC/TECHNICAL REPORT

1. PROJECT IDENTIFICATION

Award Number: DE-EE0003129
Recipient Organization: Research Foundation of CUNY on behalf of Brooklyn College
Project Title: Development of Pollution Prevention Technologies
Principal Investigators:
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Professor Roberto Sanchez-Delgado, Rsdelgado@brooklyn.cuny.edu, (718) 951-5000 x 2827
Date of Report: December 30, 2013

Federal Agency & Organization: Department of Energy – Office of the Biomass Program
Project Location: Brooklyn College

2. DISTRIBUTION LIMITATIONS

There are no distribution limitations or protected data contained in this report.

2. EXECUTIVE SUMMARY

This project investigated technologies that may reduce environmental pollution. This was a basic research/educational project addressing two major areas:
A. In the algae research project, newly isolated strains of microalgae were investigated for feedstock production to address the production of renewable fuels. An existing collection of microalgae was screened for lipid composition to determine strains with superior composition of biofuel molecules. As many microalgae store triacylglycerides in so-called oil bodies, selected candidate strains identified from the first screen that accumulate oil bodies were selected for further biochemical analysis, because almost nothing was known about the biochemistry of these oil bodies. Understanding sequestration of triacylglycerides in intracellular storage compartments is essential to developing better strains for achieving high oil productivities by microalgae. At the onset of the project there was almost no information available on how to obtain detailed profiles of lipids from strains of microalgae. Our research developed analytical methods to determine the lipid profiles of novel microalgal strains. The project was embedded into other ongoing microalgal projects in the Polle laboratory. The project benefited the public, because students were trained in cell cultivation and in the operation of state-of-the-art analytical equipment. In addition, students at Brooklyn College were introduced into the concept of a systems biology approach to study algal biofuels production.
B. A series of new nanostructured catalysts were synthesized, and characterized by a variety of physical and chemical methods. Our catalyst design leads to active nanostructures comprising small metal particles in intimate contact with strongly basic sites provided by the supports, which include poly(4-vinylpyridine), magnesium oxide, functionalized multi-walled carbon nanotubes, and graphene oxide. The new materials display a good potential as catalysts for reactions of relevance to the manufacture of cleaner fossil fuels and biodiesel, and to hydrogen storage in organic liquids. Specifically the catalysts are highly active in the hydrogenation of aromatic and heteroaromatic components of fossil fuels, the reduction of unsaturated C=C bonds in biodiesel, and the dehydrogenation of nitrogen heterocycles. In the course of our studies we identified a novel dual-site substrate-dependent hydrogenation mechanism that explains the activity and selectivity data obtained and the resistance of the new catalysts to poisoning. These results represent an important advance in basic catalytic science, regarding design and synthesis and reaction mechanisms. Additionally, this project allowed the enhancement of the laboratory facilities in the Chemistry Department of Brooklyn College for catalysis and energy research, and served as an excellent vehicle for the training of several young researchers at the undergraduate, graduate and postdoctoral level, to join the national scientific workforce.

4. COMPARISON OF ACCOMPLISHMENTS WITH GOALS AND OBJECTIVES

4.A Algal Component

Initial goals:

The hypothesis of this research project was that algal strains existed in nature that could be identified by lipid profiling for use as future platform strains for strain development in regards to renewable fuels and bioproducts generation.

The goal and objectives of this research project were completed. The goal of the algal research project was to assess newly isolated microalgae strains for potential use as high-quality feedstocks in biofuels applications. The main objective of the algae research project was the high-throughput screening of recently isolated strains of microalgae for rapid detection of cellular metabolites to be used in biofuels applications.

Actual accomplishments

A major part of this research project was the acquisition of research instrumentation. For this research an Ultra High-Performance Liquid Chromatograph (UHPLC) system the Flexar FX-15 UHPLC in combination with a Time-of-Flight_Mass Spectrometer (ToF-MS) system was acquired from Perkin Elmer. First the Tof-MS was equipped with an Electrospray Ionization (ESI) module. Within the second year of the project the Atmospheric-Pressure Chemical Ionization (APCI) probe became available. Obtaining, setup, and operation of the equipment was the first major task, which was accomplished.

4.B Catalysis Component

Initial goals:

To prepare new advanced materials composed of metallic nanoparticles immobilized on reactive basic solid supports; to explore their potential as catalysts for reactions of relevance to the manufacture of cleaner fossil fuels or of biodiesel, and understanding the underlying chemistry and reaction mechanisms. This aim is in line with the objectives of the DOE Office of Science, Catalysis Science Program, in relation to both fossil fuels and renewable fuels. The research also aligns with the Fossil Fuels Division's Fuels and Lubricants Program (Fuels Technology Subprogram, in both Advanced Petroleum-based Fuels and Non-Petroleum Based Fuels activities).

Actual accomplishments

The objectives initially proposed were met and exceeded during the time of execution of the project. In particular, the following families of catalysts were prepared, characterized and evaluated (only families 1-4 were envisaged at the onset of the project):

1. Ruthenium on poly(4-vinylpyridine), **Ru/PVPy**
2. Palladium on magnesium oxide, **Pd/MgO**
3. Ruthenium on magnesium oxide, **Ru/MgO**
4. Rhodium on magnesium oxide, **Rh/MgO**
5. Ruthenium on acid treated multi-walled carbon nanotubes, **Ru/AT-MWCNT**
6. Ruthenium on pyridine-modified multi-walled carbon nanotubes, **Ru/Py-MWCNT**
7. Ruthenium on grapheme oxide, **Ru/GO**

Apart from the catalytic studies, detailed mechanistic investigations were performed for Ru/PVPy and Ru/MgO, which allowed us to elucidate the main reaction pathways that involve novel heterolytic hydrogen activation and ionic hydrogenation of N-heterocycles, acting in concert with conventional mechanisms for plain aromatic substrates.

5. SUMMARY OF PROJECT ACTIVITIES
Below the activities of this project and the accomplishments are listed by task:

5.A Summary of Activities Algal Component
Task number: A.0.0

1. **Planned Activities**: Setup of equipment (01/01/2010-03/31/2011)
2. **Actual Accomplishments**:
   A. The setup of the UHPLC system was originally done in January 2011. The MS system was set up for use in January 2011. The UHPLC-ESI-TOF-MS was functional and in use for screening activities since January 2011. However, one additional piece of the equipment for detection of special metabolites had not been delivered yet. Perkin Elmer was behind its schedule for delivery for the APCI probe. The past variance was cured in January 2012.
   B. The new APCI probe was delivered and installed by Perkin Elmer in January 2012. At the same time software upgrades were made. The entire UHPLC system was serviced. Concurrent with the software and hardware updates to the UHPLC and TOF-MS upgrades a power stabilizing system was added to the system to provide stabilized power supply to the UHPLC TOF-MS system. The instrument is now complete.
   C. The PI Dr. Polle and the graduate student Mr. Neofotis received a four day on-site training at the Perkin Elmer facility in Connecticut in January. This training focused on optimizing use of the UHPLC system and on optimizing use of the new APCI probe.
3. **Explanation of Variance**: No variance.
4. **Outcome**: Activities completed

Task number: A.1.0

1. **Planned Activities**: Hiring of personnel (09/01/10 - 06/30/13)
2. **Actual Accomplishments**:
   Originally, Ms. Cassana Fisher-Ramos was recruited as a Masters student associated with the project starting on 10/01/2010. She left the lab in 2011. At that time, the Ph.D. student Mr. Peter Neofotis replaced the Masters student Ms. Fisher-Ramos.
   Mr. Florenal Joseph was hired as a Laboratory Research Associate starting on 11/01/2010. He continued on this project through 09/21/12. Mr. Florenal Joseph left the project in September 2012 to advance his career. The Ph.D. student Mr. Neofotis took over the responsibilities of Mr. Joseph. Mr. Peter Neofotis continued on this project through 03/31/13. Mr. Neofotis training was extended to prepare him to replace Mr. Joseph. Mr. Neofotis training was focused on biochemistry and use of the UHPLC-TOF-MS instrument to replace Mr. Joseph in the operation of the instrument. Mr. Neofotis continued to work on cellular fractionation and strain analysis until September 2013.
   Four undergraduate students (Mr. Hwang, Mr. Banniattis, Ms. Crown, Ms. Badiner) were incorporated to the project as research assistants throughout the Fall 2010. The undergraduate students Mr. Banniattis and Ms. Crown graduated in May 2011. They were replaced on the project by two new students Mr. Malkin and Ms. Lyristis. One new student Mr. Louisia, was introduced to algal cultivation and he worked with Mr. Joseph on growth analysis of algal strains. Two new undergraduate students (Ms. Kulichenkova and Mr. Hasan) joined the laboratory. Both students began their training in metabolite biochemistry. In addition, several work-study students joined the laboratory and some of them were trained to support the laboratory on future strain cultivation.
   Mr. Huang continued to work with Mr. Neofotis. Mr. Huang continued to receive training for the UHPLC-TOF-MS instrument and in data analysis. Four new undergraduate

students joined this laboratory in January 2013. The students received general training by the PI and by Mr. Neofotis.The undergraduate students Mr. Malkin, Mr. Banniattis, and Ms. Badiner moved on to a different project in this laboratory. The undergraduate students Mr. David Rosenberg and Ms. Irina Kulichenkova who had joined this laboratory in September left this laboratory. The work-study students who had joined the laboratory in September could not continue their work in the laboratory. Several new undergraduate students joined the laboratory in June 2013. One new undergraduate student joined the lab in August 2013.

3. **Explanation of Variance**: No variance.
4. **Outcome**: Activities completed.

Task number: A.2.0

1. **Planned Activities**: Cultivation of strains of microalgae was an ongoing activity.
2. **Actual Accomplishments**:
   About 4,000 strains were maintained by the lab personnel. Some of the best performing strains from the bubbling columns were analyzed regarding their lipid profile.
3. **Explanation of Variance**: No variance
4. **Outcome**: Activities completed.

Task number: A.3.0

1. **Planned Activities**: Metabolite extraction for strains was an ongoing activity.
2. **Actual Accomplishments**:
   - Following the on-site training at Perkin Elmer the extraction and analysis method for lipids and carotenoids was updated in January 2013. Following separation by reverse-phase liquid chromatography, the analysis of extracts then included a first characterization of pigments through the Photodiode Array Detector (PDA), which was directly followed by injection into the APCI probe with characterization of molecules by the TOF-MS.
   - The new protocol using the APCI probe was used.

3. **Explanation of Variance**: No variance
4. **Outcome**: Activities completed.

Task number: A.4.0

1. **Planned Activities**: Biomass analysis (09/01/2010-09/30/2013)
2. **Actual Accomplishments**:
   Biomass analysis was performed this quarter.
3. **Explanation of Variance**: No variance.
4. **Outcome**: Activities completed.

Task number: A.5.0

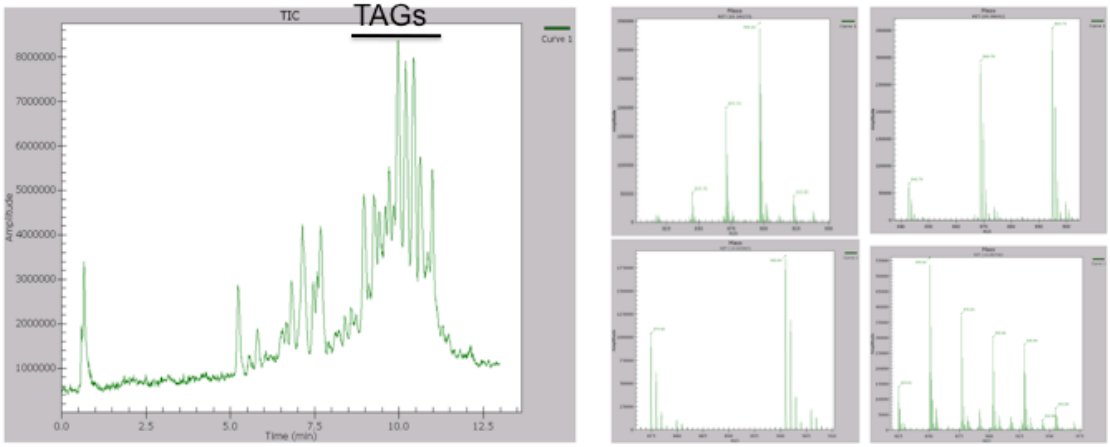
1. **Planned Activities**: Identification of strains was an ongoing activity.
2. **Actual Accomplishments**:
   - In the first year of the project, one saline water strain of microalgae belonging to the green algae (*Dunaliella salina* CCAP19/18) had been identified with relatively high content of lipids Cells of strain *D. salina* CCAP19/18 were fractionated and lyophilized for further analysis. Lyophilized samples were sent out to PNNL for proteomic analysis.

     Figure 1: An exemplary analysis of biomass and lipids of the green alga *Dunaliella salina* strain CCAP19/18 is shown in the figure below. A lipid profile taken in the positive ESI mode is shown indicating a number of different triacylglycerol classes present in this alga.

Task Identification of a promising algal strain.

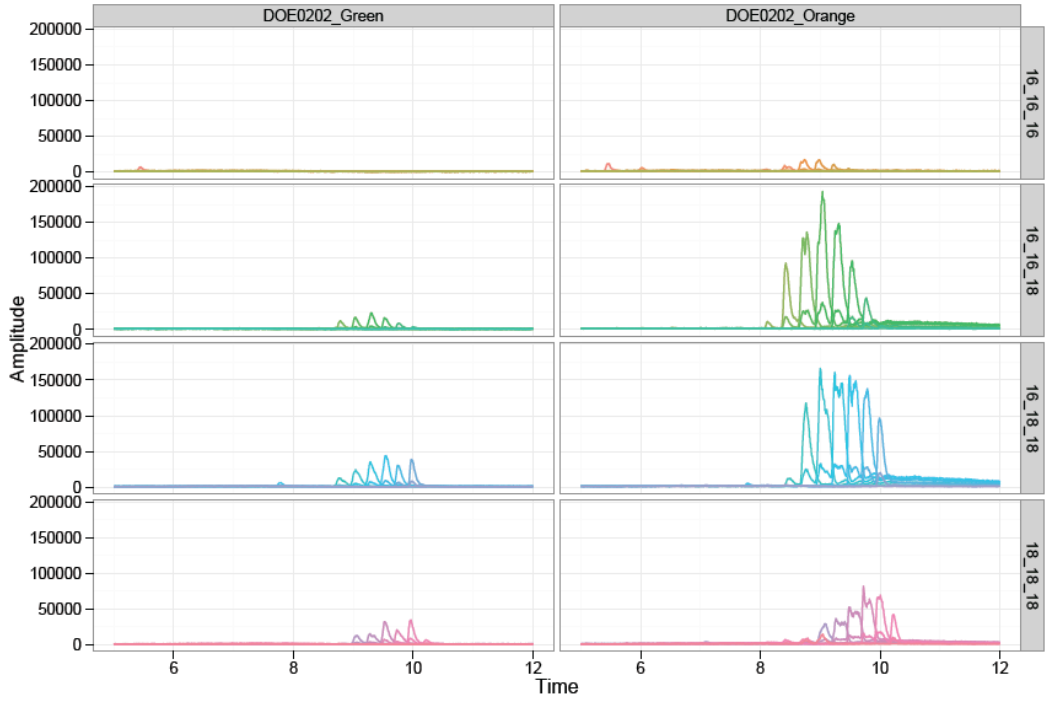
The unicellular green alga *Dunaliella salina*

Strain	Neutral Lipids	Polar Lipids	Total Lipids
Dunaliella salina	*15.9%*	*23.7%*	*39.6%*



- In the first quarter of the second year several more strains of the marine diatom *Amphora sp.* were identified as a high triacylglycerol accumulating species. Several strains were investigated regarding biomass and lipid productivity and will be used for oil body isolation The first cell fractionations of strains of the diatom genus *Amphora* did not result clean oil bodies.
- Growth of biomass to repeat fractionation of cells was begun again for the strain *Amphora sp.* EN0743. In the Q4 of 2012 a protocol was established to isolate cell fractions for *Amphora sp.* EN0743.
- In the second year several further marine strains, possibly of the lineage of Eustigmatophytes, were determined to contain lipids. These strains were not yet used for further analysis.
- Some freshwater strains of green (examples are EN1234. EN1235, DOE0202) algae possibly belonging to the genus *Chlorococcum,* were identified as oil producers. However, the thick cell wall of these strains prevents cellular fractionation of these strains for oil body isolation.

Figure 2: Shown is a comparison of the profiles of triacylglycerol classes from extracts of non-stressed (green) versus stressed (orange) cells of the green algal strain DOE0202 containing either 3xC16 fatty acids, 2xC16 & 1xC18, 1xC16 & 2xC18, or 3xC18 fatty acids. Orange cells contained large amounts of triacylglycerols, but ultimately cells could not be fractionated due the presence of thick and tough cell walls.



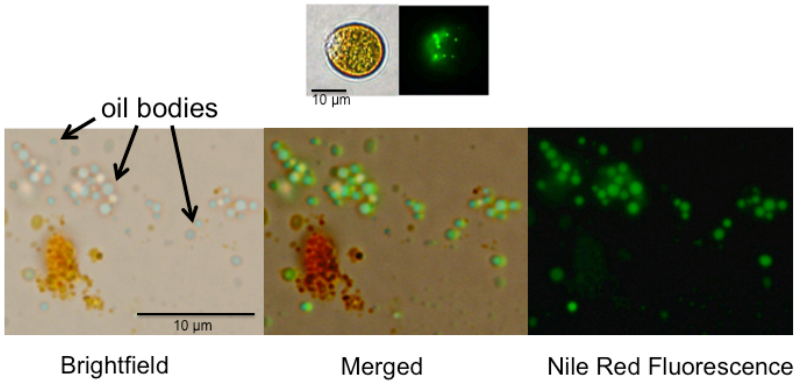
- Two more freshwater strains (EN0004 & DOE0152) belonging to the species *Scendesmus obliquus* were identified as high biomass and lipid producers. Again clean cell fractionation could not be achieved for the *Scenedesmus* strains, due to the presence of a tough cell wall. Consequently, no oil bodies could be isolated from these strains.
- One more marine diatom *Amphora* strain DOE0938 was identified for cellular fractionation and isolation of oil bodies.

3. **Explanation of Variance**: Positive variance. More strains than originally anticipated were identified from several algal lineages. At least four freshwater strains were identified for cellular fractionation. In addition, several salt-water strains were identified as promising strains for cellular fractionation.
4. **Outcome**: Completed activities.

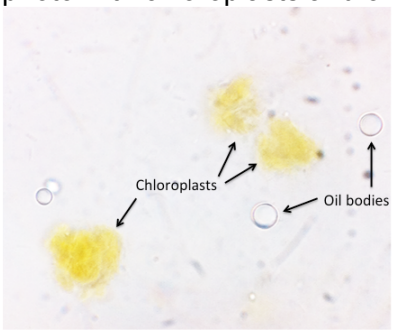
Task number: A.6.0

1. **Planned Activities**: oil body isolation (07/01/2012-09/30/2013)
2. **Actual Accomplishments**:
   - In the Q3 of 2012 using the newly established protocol, cellular fractions including oil bodies were isolated for *D. salina*. Samples with cellular fractions were sent to a collaborator at PNNL for further analysis of the proteins in the fractions.
     Figure 3: Shown are photos taken of cellular fractions in the process of oil body isolations. Oil bodies could be labeled and visualized with the fluorescent dye Nile Red.

Task Oil body isolation from the alga *Dunaliella salina.*



- In the Q3 of 2012, of the strain *Amphora sp.* EN0743 was examined for isolation of oil bodies. Several different types of methods to break cells were determined (including sonication, osmotic shock, and French Press application). Originally, none of these methods resulted in isolation of clean oil bodies. In the Q4 of 2012 new cultures of strain *Amphora sp.* EN0743 were used for development of a cell fractionation protocol. Oil bodies can now be obtained from this strain of *Amphora*. **Figure 4:** Cell fractionation of the diatom *Amphora sp.* strain EN0742. Shown is a photo with chloroplasts and oil bodies.



- The promising strains of *Chlorococcum* could not be used for cellular fractionation, due to the thick walls of cells that accumulated the oil bodies.
- Cells of *Scenedesmus obliquus* also proved to be difficult to break. Several cultures of about 300 mL were grown in bubbling columns to obtain growth curves and biomass from different growth phases. Metabolite extractions were possible, but high quality subcellular fractionation was not achieved.
- In the Q2 of 2013 a focus was placed on repeating the cellular fractionation of the green alga *Dunaliella salina* and of the diatom *Amphora* to improve the quality of the cellular fractionations. The amount and quality of material for analysis of the cellular fractions of *Dunaliella salina* could be increased. Oil bodies could be isolated from the diatom Amphora, but the quantity still has to be increased.
- In the Q3 of 2013 cellular fractionation of the green algal strain *D. salina* CCAP19/18 was repeated to obtain new material for repeated proteomic analysis. In addition, the diatom Amphora strain EN0742 regrown and cellular fractionation repeated with a concomitant increase in the quantity of oil bodies. Sufficient quantities of oil bodies could be isolated.

3. **Explanation of Variance**: No variance.
4. **Outcome**: Activities completed.

Task number: A.7.0

1. **Planned Activities**: Extraction of metabolites and proteins from oil bodies (10/01/2012-09/30/2012).
2. **Actual Accomplishments**:
   - For the alga *Dunaliella salina*, the sample preparation/processing for protein identification by mass spectrometry was completed. Samples were sent out to PNNL for proteomic analysis. For *D. salina* the method of oil body isolation was repeated to obtain more material for a better analysis.
   - Extraction of metabolites was successful for different cellular fractions including oil bodies for the diatom *Amphora sp.* EN0743. A second strain of Amphora DOE0738 was used successfully to isolate oil bodies. However, not enough material was obtained for further analysis.
   - In Q3 of 2013 the sample preparation was repeated for the green algal strain *Dunaliella salina* CCAP19/18 and the diatom *Amphora sp.* strain EN0742. The quality and the quantity of material for analysis was improved. SDS-gel electrophoresis confirmed that enough protein material was extracted from cellular fractions.
3. **Explanation of Variance**: Cured.
4. **Outcome**: Task completed.

Task number: A.8.0

1. **Planned Activities**: Analyze the metabolites from oil bodies (04/01/2013-09/30/2013)
2. **Actual Accomplishments**:
   - For *Dunaliella salina* cellular fractions were analyzed for triacylglycerol contents. Differences were identified between the profiles of total cell extracts to oil bodies.
   - For a second species, the diatom *Amphora sp.,* extracts of cellular fractions were obtained and analyzed regarding lipids.
   - In Q3 of 2013 experiments were repeated with concomitant metabolite analysis by Photo Diode Array for pigment analysis and APCI-MS for lipid analysis.
3. **Explanation of Variance:** Cured.
4. **Outcome:** Task completed.

Task number: A.9.0

1. **Planned Activities:** Comparison of the protein profiles from whole cells to those obtained from oil bodies. (04/01/13-09/30/13)
2. **Actual Accomplishments:**
   - Samples from *D. salina* were obtained. We received the first data on proteins from oil bodies in January 2013. Analysis of the protein sequences was done based on genomic sequencing data available to the Polle laboratory. Several proteins could be identified. Several of the proteins for which sequences were obtained, were of unknown function. In Q2 of 2013 SDS-Page Electrophoresis was performed on cellular fractions to investigate differences between cellular compartments. It was found that the fractionation protocol used lead to enrichment of a number of proteins that were present in oil bodies. In Q3 of 2013 new samples were prepared with more protein material for proteomic analysis. The samples were submitted to the Rockefeller University Proteomics facility for analysis. These new samples provided sufficient material for higher quality proteomic results regarding proteins present in enriched oil bodies. This new proteomic analysis showed presence of specific oil body proteins confirming the high quality of the samples. The analysis of the proteomic results showed that only about 5% of all the MS spectra could be matched to peptides. Only when our high quality transcriptomic data from *D. salina* was used

for the peptide matching, about 60 % of all the MS spectra could be matched to peptides. Our results demonstrated that high quality proteomic analysis requires high quality genomic and/or transcriptomic data that can be used to identify proteins.

- In Q3 of 2013 cellular fractions from the diatom Amphora sp. strain EN0742 were obtained. Analysis by SDS-Page Electrophoresis showed that the fractionation protocol used lead to enrichment of a number of proteins that were present in oil bodies. A sample with enriched oil bodies was submitted for proteomic analysis to the Rockefeller University Proteomics facility. The number of proteins recognized was relatively low due to the absence of genomic/transcriptomic data for this species. However, a number of cytoskeletal proteins were identified present in high abundance in the enriched oil body fraction. Also, a variety of unknown proteins were identified.

3. **Explanation of Variance:** Cured.
4. **Outcome:** Task completed.

6.A PRODUCTS DEVELOPED UNDER THE AWARD (ALGAL COMPONENT)

Task number: A.10.0

1. **Planned Activities**: Quarterly and annual reporting on results. Dissemination of results by presenting at scientific conferences and publication of results.

2. Actual Accomplishments:

Summary: All reports were submitted on time. Results were disseminated as oral presentations and/or posters. One manuscript on algae isolation and screening was submitted for publication as a book chapter in October 2013.

- Quarterly report for period September 2010 submitted in October 2010.
- Quarterly report for period 10/01/2010 to 12/31/10 submitted in January 2011.
- Quarterly report for period 01/01/11 to 03/31/11 submitted in April 2011.

Attended the DOE review meeting in March 2011 in Annapolis and reported on the status of the project. Answered to the reviewer's questions by submitting a final report for the review meeting.

Attended the JGI user meeting in Walnut Creek, CA in March 2011 and presented a poster on results of estabilishing a protocol of oil body isolation and lipid profiles of the species *Dunaliella salina*.

Title: Lipidomics of the Unicellular Green Alga *Dunaliella salina*.

Authors: Polle J.E.W.1*, Joseph F.1, Fisher-Ramos C.1, Samburova V.2, Zielinska B. 2, Lemos M.S. 3, Hiibel S.R.3, and Cushman J.C.3

1 Brooklyn College, The City University of New York. Brooklyn, New York, USA.

2 Desert Research Institute, 2215 Raggio Pkwy, Reno, NV 89511, USA

3 Department of Biochemistry and Molecular Biology, University of Nevada, Reno, Nevada, 89557, USA

- Quarterly report for period 04/01/11 to 06/30/11 submitted in July 2011.

Presented results in an oral presentation at the 3rd Annual Algae World Summit 2011 in May in San Diego, CA.

Title:"Microalgae strain development for biofuels applications."

Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.

- Quarterly report for period 07/01/11 to 09/30/11 submitted in October 2011.

Presented results in an oral presentation at the First International Conference on Algal Biomass, Biofuels, and Bioproducts in July in St Louis, MO.

Title:"Identification of novel strains of microalgae for biofuels applications."

Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.

- Quarterly report for period 10/01/11 to 12/31/11 submitted in January 2012.
  Presented results at Hofstra University in NY in November 2011 in an oral presentation.
  Title "The Potential of Microalgae for Biofuels Applications: Identification of novel strains of algae."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
- Quarterly report for period 01/01/12 to 03/31/12 submitted in April 2012.
  Peer reviewed publication:
  Lohr M, Schwender J, and JEW Polle (2012) Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae. Plant Science 185-186: 9-22.
  Invited talks given:
  1. University of Illinois at Urbana-Champaign, IL, USA
  "Microalgae Biofuels: Strain Selection and Metabolism."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
  2. Brooklyn College of CUNY, Brooklyn, NY, USA
  "Microalgae strain development: From cell isolation to cell factories."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
  3. Conference on Genomics & Systems Biology at NYU Abu Dhabi, Abu Dhabi
  "Strain Development of Microalgae: From strain isolation to cell factories."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
- Quarterly report for period 04/01/12 to 06/30/12 submitted in July 2012.
  No peer reviewed publication in this reporting period.
  Several invited talks were given:
  1. Rutgers University in New Brunswick, NJ, USA
  "Microalgae Biofuels: From Bioprospecting to Cell Factories"
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
  2. 9th European Workshop on Biotechnology of Microalgae, Nuthetal, Germany
  "Discovery and Development of Strains of Microalgae for Phototrophic Biofuels Applications."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
  3. 2nd Algal San Diego, CA, USA
  "Isoprenoid metabolism in microalgae: Biosynthesis, regulation, and metabolic sinks."
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
- Quarterly report for period 07/01/12 to 09/30/12 submitted in October 2012.
  One invited talk was given in this reporting period:
  1. 6th Annual Algae Biomass Summit 2012, Denver, CO, USA, September 2012,
  Title: "Isoprenoid Metabolism in the Diverse Lineages of Microalgae".
  Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.
- Quarterly report for period 09/01/12 to 12/31/12 submitted on time in January 2013.
- Quarterly report for period 01/01/13 to 03/31/13 submitted on time in April 2013.

One manuscript published in the journal 'Current Opinion In Chemical Biology' (2013) 17, 1-9:

Title: "Metabolic and Cellular Organization in Evolutionarily Diverse Microalgae As Related to Biofuels Production."

Authors: Mark Hildebrand, Raffaela M. Abbriano, Juergen E. W. Polle, Jesse C. Traller, Emily M. Trentacoste, Sarah R. Smith, and Aubrey K. Davis

The second submitted manuscript on algal strain isolation screening and characterization was rejected by the journal 'Algal Biology'

No presentations given in this quarter.

- The quarterly report for the period 01/04/13 to 06/30/13 was submitted on time.

One invited, oral presentation was given at the European Algae Biomass Conference in Vienna, Austria:

Title: "Bioprospecting Microalgae for Biofuels & Bioproducts: Finding, Screening, and Analysis of Strains.

Author: Polle J.E.W., Brooklyn College, The City University of New York. Brooklyn, New York, USA.

The Bioenergy Technologies Office (BETO) 2013 Project Peer Review was attended in May 2013 and a presentation was given in the Biodiesel section.

- The final report including period 07/01/13 to 09/30/13 submitted on time in October 2013.

No presentations were given in Q3 of 2013.

One manuscript with the title "Microalgae strain isolation, screening, and identification for biofuels and high value products" for the book 'Micro-Algal Production for Biomass and High-Value Products', Eds. Slocombe & Benemann, ISBN: 978-1-4822-1970-8 (Taylor & Francis Catalog #:K22169) is *in review*.

3. **Explanation of Variance**: No variance
4. **Outcome**: Task completed.

5.B Summary of Activities Catalysis Component

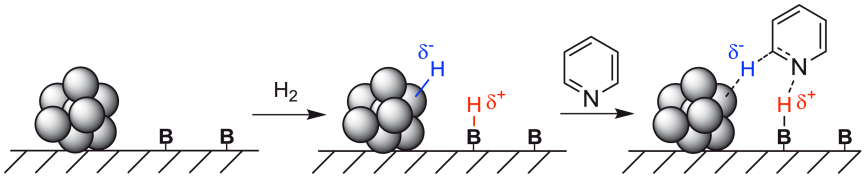
5.B.1. Background

Fossil fuels—coal, oil and natural gas—currently provide over 80% of the energy consumed in the US and will continue to be the predominant energy source worldwide by 203, in spite of the aggressive development of alternative renewable energy sources. In particular, global consumption of conventional transportation fuels will continue to grow. Environmental regulations impose increasingly severe restrictions on the aromatics, sulfur, and nitrogen content allowed in fuels; the low levels required are difficult to achieve with current refining technologies. Considering the current level of oil consumption, in the foreseeable future liquid fuels will necessarily come from unconventional sources, such as heavy and extra-heavy oil and bitumen deposits. Such fossil materials contain higher levels of polycyclic aromatics, nitrogen and sulfur, all of which need to be removed or transformed to produce cleaner fuels and to avoid poisoning of precious metal catalysts employed in the refining process [1-3].

Catalytic hydrogenation plays a pivotal role in the manufacture of cleaner fuels: the proportion of aromatic hydrocarbons is lowered through hydrogen addition; removal of nitrogen and sulfur is achieved through hydrodenitrogenation (HDN) and hydrodesulfurization (HDS), both of which involve key hydrogenation steps within complex reaction networks. Conventional metal sulfide hydrotreating catalysts (Co-Mo, Ni-Mo and Ni-W) can only saturate a moderate proportion of aromatics due to thermodynamic equilibrium limitations under typical operating conditions (300–400 °C, 50–100 atm). Supported noble metal catalysts, on the other hand, can function at lower temperatures far from equilibrium conditions but they are easily poisoned by N- and S-containing compounds present in refinery feeds. *Therefore, there is a continuing need for efficient catalysts capable of promoting hydrogenation of aromatics under moderate reaction conditions, while being resistant to poisoning by nitrogen and sulfur species* [3-4].

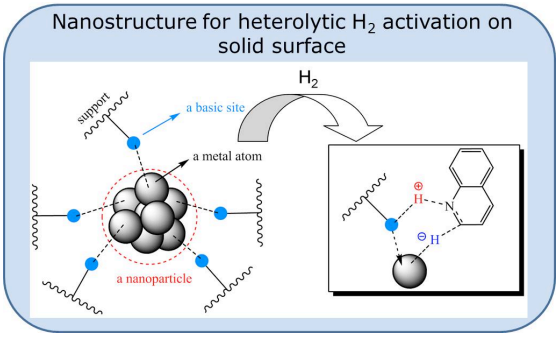
Although hydrogenation by metals is a well-developed field, the use of nanoparticles (NPs) in catalysis is attracting increasing attention due to the advantageous activity and selectivity features associated with the small particle size. Metal nanoparticles (MNPs) stabilized by ligands or macromolecules, surfactants, ionic liquids, or supports, have been employed for hydrogenation of aromatics, mostly of benzene and monocyclic arenes. The role of the solid supports, in most instances, is to avoid NP aggregation and to facilitate catalyst recovery and recycling, but seldom to intervene directly in the key steps of the reaction [5].

The most widely accepted hydrogenation mechanisms on solid metal catalysts involve homolytic H_2 splitting on surface metal sites into hydrogen atoms, which are transferred to the chemisorbed substrates. N- or S-containing species frequently bind too strongly to such active metal sites, causing catalyst deactivation or poisoning. An interesting but much less frequently encountered alternative pathway is the base-assisted heterolytic splitting of hydrogen into H^- and H^+, followed by outer sphere ionic transfer to polar unsaturated bonds of the substrate (Scheme 1). Mechanisms of this type do not require binding of substrates or products to the metal sites, thereby opening a possibility to avoid poisoning. *Ionic hydrogenation routes are well established in solution chemistry but they remain largely unexplored on solid catalysts, although such reaction pathways have been suggested to occur on metal sulfides or ceria-supported gold catalysts, with surface sulfur or oxygen sites acting as the proton acceptors, respectively.*



Scheme 1. Heterolytic H_2 activation and ionic transfer to polar unsaturated bonds on a solid catalyst surface comprising metal NPs and basic functionalities (B) on the support.

Our working hypothesis is that a surface nanostructure composed of small metallic particles immobilized on a support that is rich on basic sites can promote heterolytic hydrogen splitting and ionic hydrogenation mechanisms through a metal-support bifunctional effect.



During the course of this project we have prepared a number of catalysts based on a design that responds to our working hypothesis, evaluated them in hydrogenation /dehydrogenation reactions of relevance to energy and fuels issues, and studied the most important reaction mechanisms, as described below:

5.B.2. Catalyst synthesis

The most useful general synthetic method developed in this project is the chemical reduction of appropriate metal salts with sodium borohydride in the presence of the support suspended in methanol, as exemplified for the Ru/PVPy catalyst in Eq. 1:

$$RuCl_3 \cdot 3H_2O + NaBH_4 \xrightarrow[MeOH,\ r.t.]{P4VPy\ (2\%\ cross\text{-}linked)} Ru(0)/P4VPy \qquad (1)$$

This procedure was applied, with minor individual adaptations, to the preparation of all the catalysts produced in this project, listed below:

1. Ruthenium on poly(4-vinylpyridine), **Ru/PVPy**
2. Palladium on magnesium oxide, **Pd/MgO**
3. Ruthenium on magnesium oxide, **Ru/MgO**
4. Rhodium on magnesium oxide, **Rh/MgO**
5. Ruthenium on acid treated multi-walled carbon nanotubes, **Ru/AT-MWCNT**
6. Ruthenium on pyridine-modified multi-walled carbon nanotubes, **Ru/Py-MWCNT**
7. Ruthenium on grapheme oxide, **Ru/GO**

The materials were prepared in high yields in sufficient quantities for characterization and evaluation purposes. The method is reliable, easily reproducible, technically simple, and based on commercially available materials. Details available in our publications [7-9].

5.B.3. Catalyst characterization

All new materials were thoroughly characterized prior to their use in catalytic tests, by a combination of methods that include TEM, XRD, XPS, and surface analysis. Typical data for Ru/MgO, one of the most efficient catalysts prepared, is shown in Fig. 1 below. The complete data for all new materials is available in the publications resulting from this project [7-9], or upon request to our laboratory.

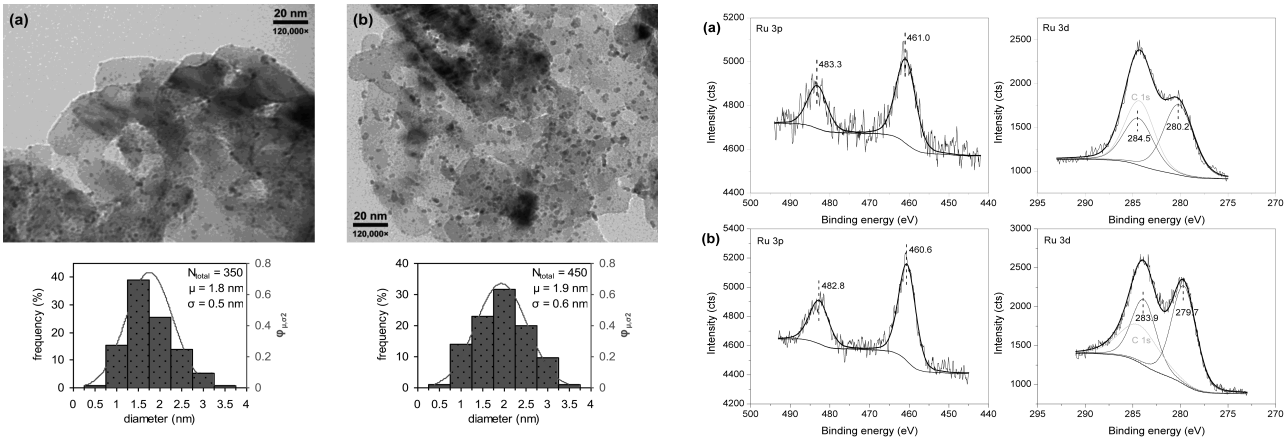


Fig. 1. TEM and XPS analysis of Ru/MgO catalyst

5.B.4. Hydrogenation catalysis

All the catalysts prepared were evaluated in the hydrogenation of arenes, as well as N- and S-heteroaromatic compounds, representative of fossil fuel components. Most of the catalysts investigated displayed high activity (measured in turnover frequencies, TOF) accompanied either by high versatility or selectivity. Fig. 2 summarizes the behavior of 5% Ru/MgO toward a wide variety of substrates, which is superior to other known catalysts of this type, and could be of use in the development of practical hydrotreatment catalysts. Details in our publications [7-9].

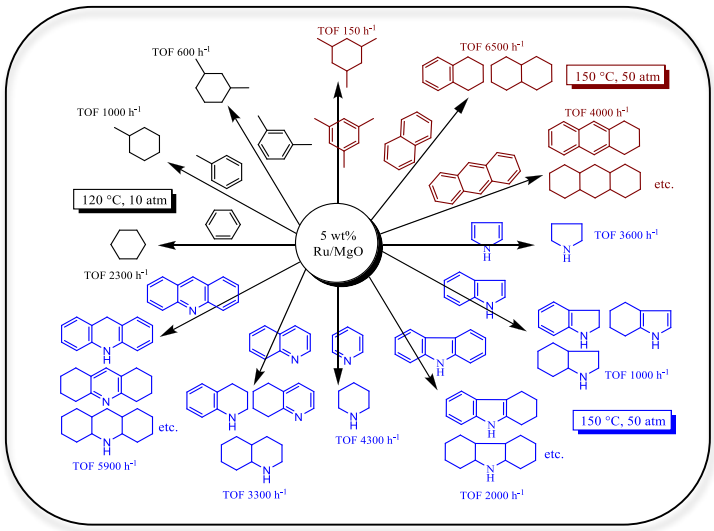


Fig. 2. Catalytic properties of Ru/MgO in aromatic hydrogenation

The Pd/MgO analogous system is less active but shows a high selectivity toward N-heterocycles and is also very active in the partial hydrogenation of biodiesel under extremely mild conditions (Fig. 3). Partial saturation of the C=C bonds of biodiesel results in improved stability and combustion properties. Results published in 2012 [8].

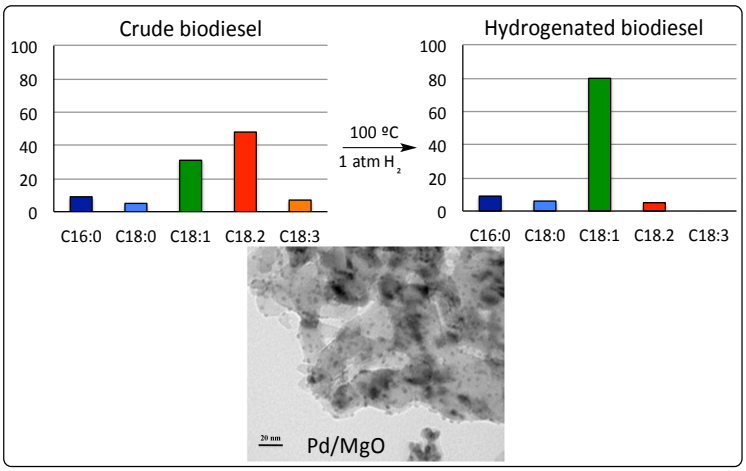


Fig. 3. Catalytic partial hydrogenation of biodiesel by 1% Pd/MgO

5.B.5. Mechanistic studies in hydrogenation

Extensive studies were conducted in order to elucidate the principal reaction mechanisms and to test our hypothesis of promoting heterolytic hydrogen splitting and ionic mechanism by our metal/support catalyst design. These included competition experiments, selective poisoning, effects of solvents and additives, and characterization of used catalysts. Our most important discovery is that indeed our catalyst design leads to the formation of two distinct active sites A and B. Sites type A promote novel reaction mechanisms in the case of N-heteroaromatic compounds, that proceed through heterolytic/ionic pathways (a), practically unprecedented in the literature. Simultaneously, type B sites follow the more conventional hemolytic pathways (b). A summary of our mechanistic model is presented in Fig. 4 for the Ru/PVPy catalyst (published in 2011 [7]) and we have demonstrated a similar set of reactions for Ru/MgO [9], which allows us to safely assume that similar mechanisms are operative for all our catalysts.

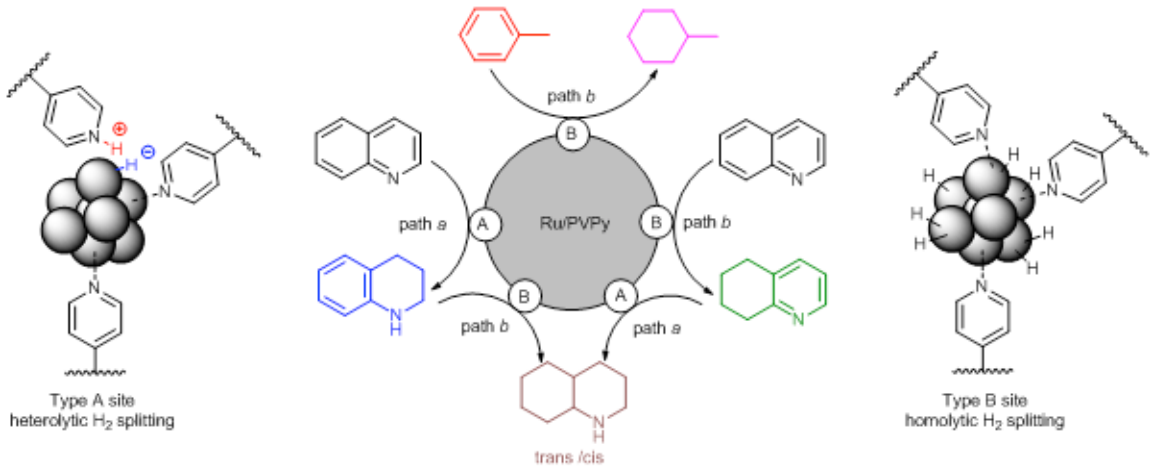


Fig. 4. A novel dual-site substrate-depended hydrogenation mechanism was discovered

5.B.6. Catalytic dehydrogenation of N-heterocycles

This aim was not contemplated in our original objectives. However, once we established the efficacy of our new catalysts for the hydrogenation of N-heteroaromatics, we realized that the principle of microscopic reversibility dictates that the reverse reaction must be accessible under similar reaction conditions. A hydrogenation/dehydrogenation cycle of this type would constitute a method of hydrogen storage in organic liquids, which would be a simple alternative to the design of special solid materials for hydrogen storage [10]. Additionally, this type of technology would make use of the available infrastructure for storing and transporting liquid fuels.

Several of our catalysts can perform these transformations under moderate reaction conditions and at this point the most efficacious systems appear to be those composed of Ru nanoparticles on carbon nanomaterials, more specifically on pyridine-modified multiwalled carbon nanotubes [11]. This application is summarized in Fig. 5.

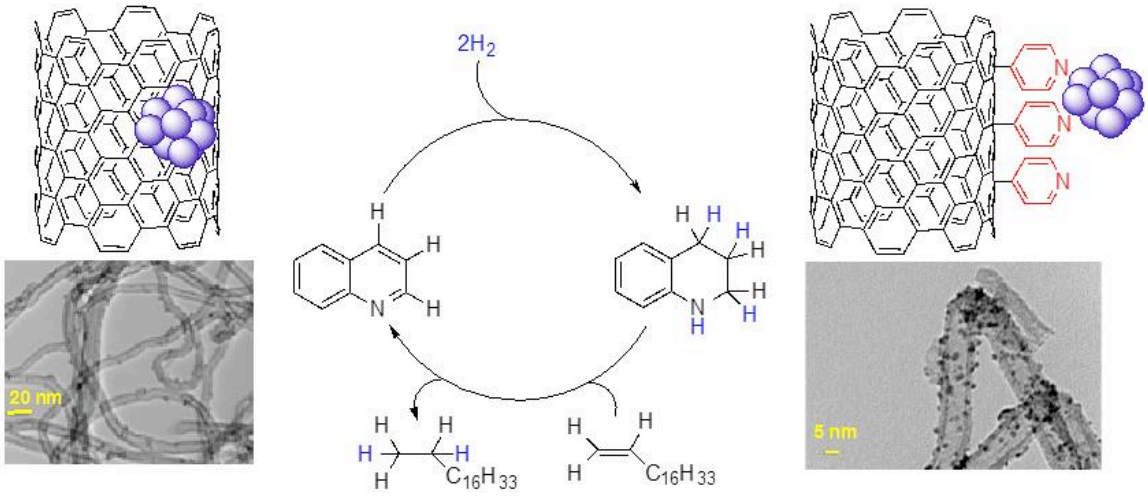


Fig. 5. Hydrogenation/dehydrogenation of N-heterocycles promoted by our Ru/Py-MWCNT catalyst could lead to a new technology for hydrogen storage in organic liquids.

5.B.7 Bibliography

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6.B PRODUCTS DEVELOPED UNDER THE AWARD (CATALYSIS COMPONENT)

Publications (in reversed chronological order)
Funding from the DOE was acknowledged in all these publications

1. A. Sánchez, M. Fang, A. Ahmed, and R. A. Sánchez-Delgado*, "Hydrogenation of arenes, N-heteroaromatic compounds, and alkenes catalyzed by rhodium nanoparticles supported on magnesium oxide" **2013** (submitted to Applied Catalysis)

2. M. Fang and R. A. Sánchez-Delgado* "Ruthenium nanoparticles supported on magnesium oxide: a versatile and recyclable dual-site catalyst for hydrogenation of mono- and poly-cyclic arenes, N-heteroaromatics, and S-heteroaromatics", **2013** (accepted for publication in the Journal of Catalysis)

3. R. Rahi, M. Fang, A. Ahmed and R. A. Sánchez-Delgado*, "Hydrogenation of quinolines, alkenes, and biodiesel by palladium nanoparticles supported on magnesium oxide." *Dalton Transactions* **2012**, *41*, 14490-14497.

4. M. Fang, N. Machalaba and R A. Sánchez-Delgado,* "Hydrogenation of arenes and N-heteroaromatic compounds over ruthenium nanoparticles on poly(4-vinylpyridine): a versatile catalyst operating by a substrate-dependent dual site mechanism" *Dalton Transactions* **2011,** *40***,** 10621–10632.

Conference presentations (only abstracts, not proceedings)
Funding from the DOE was acknowledged in all these presentations

1. R. Rahi and R. A. Sanchez-Delgado, "Hydrogenation of Aromatic Compounds by Metal Nanoparticles on Basic Supports"*,* Spring National Meeting of the American Chemical Society, 2010

2. R. Rahi and R. A. Sanchez-Delgado, "Hydrogenation of Aromatic Compounds by Metal Nanoparticles Supported on Basic Supports"*,* Gordon Research Conference in Inorganic Chemistry, 2010

3. R. Rahi and R. A. Sanchez-Delgado, "Hydrogenation of Aromatic Compounds by Metal Nanoparticles on Basic Supports"*,* Middle Atlantic Regional Meeting of the American Chemical Society, 2011

4. M. Fang and R. A. Sánchez-Delgado, Ruthenium nanoparticles supported on poly(4-vinylpyridine) as catalyst for hydrogenation of aromatics ad evidence for a dual site mechanism, Middle Atlantic Regional Meeting of the American Chemical Society, 2011

5. M. Fang and R. A. Sánchez-Delgado, Ruthenium nanoparticles supported on poly(4-vinylpyridine) as catalyst for hydrogenation of aromatics, Gordon Research Conference on Inorganic Chemistry, 2011

6. R. Rahi, A. Ahmed and R. A. Sanchez-Delgado, "Hydrogenation of aromatic compounds and biodiesel by palladium nanoparticles supported on magnesium oxide"*,* Gordon Research Conference in Inorganic Chemistry, 2010

7. D. Vovchok, A. Sánchez and R. A. Sánchez-Delgado, Hydrogenation of aromatic compounds by ruthenium nanoparticles on acid-treated multi-walled carbon nanotubes, The New York Undergraduate Research Conference of the American Chemical Society

8. A. Sánchez and R. A. Sánchez-Delgado, “Hydrogenation of alkenes, arenes and heteroaromatic compounds catalyzed by rhodium nanoparticles on magnesium oxide”, Gordon Research Conference on Catalysis., 2012

Prof. R. A. Sánchez-Delgado *presented the following invited lectures containing the results of the project and acknowledging funding from the DOE:*

1. "Nanostructured Hydrogenation Catalysts: Novel Reaction Pathways of Relevance to the Manufacture of Cleaner Fuels." Nanotechnology Workshop. CUNY Graduate Center. New York, November 2012.

2. "Catalytic Hydrogenation and Related Reactions of Relevance to the Manufacture of Cleaner Fossil Fuels." Earth & Environmental Engineering Department, Columbia University. New York, Oct. 2011.

3. "Ruthenium in Medicinal and Catalytic Applications." Chemistry Department, Pace University. Nov. 23. 2010.

Persons trained through this project:

1. Dr. Antonio Sánchez, Postdoctoral Research Associate of the project Currently employed as R&D Chemist at Air Liquide/Voltaix, New Jersey, involved in developing new materials for semiconductor applications.

2. Dr. Minfeng Fang, PhD student of the project, graduated March 2013. Currently continues as postdoctoral research associate at Brooklyn College, seeking permanent employment in National Lab or company.

3. Ms. Reena Rahi, PhD student of the project. Currently writing-up her doctoral dissertation to defend in early 2014. She intends to seek a postdoctoral position and later an industrial research position.

4. Mr. Atif Ahmed, undergraduate student of the project graduated May 2013. Currently employed as Flavor Chemist in Training at Virginia-Dare, a Brooklyn-based flavor and fragrance company.

5. Mr. Dimitriy Vovchok, undergraduate student, graduated May 2013, currently pursuing PhD studies in Chemistry and Materials Science at SUNY Stony Brook.