Pakistan-U.S. Science and Technology Cooperation Program
Annual Technical Report Form

Reports should be prepared jointly by the Pakistani and U.S. principal investigators and should cover all project-related activities carried out during the reporting period on both sides. Please expand the boxes below to accommodate all the information you need to include.

Project Title: Solid Waste Management for Bioenergy Production	
Pakistani Principal Investigator: Romana Tabassum, Ph.D.	**U.S. Principal Investigators**: William Orts, Charles Lee, Kevin Holtman
Reporting Period: October 1, 2011 – September 30, 2012	

Summary of Activities During Reporting Period:

This program was initiated during the first quarter of 2012. More specifically, the project agreement was signed in December 2011. The funds were released by HEC to Professor Tabassum in February, 2012 with a separate account opened at NIBGE after receiving the funds. Research was started in March, 2012. Professor Tabassum is actively purchasing several items that are critical for the success of this joint project, as listed in the proposal. The most significant is design and installation of a $50M^3$ anaerobic digestor. Final specifications were established by consulting with Dr. Kevin Holtman of the USDA-Albany lab, one of USDA's experts on anaerobic digestion. Dr. Holtman has experience in scale-up of this equipment. For this purchase an open tender was listed in the National newspaper and NIBGE web site in July 2012. The technical evaluation has been made and the order will be issued after completion of procedures by the procurement branch of NIBGE in Pakistan. The anaerobic digester of 50M 3 has been designed for agricultural wastes, fruits, and vegetable wastes after a long survey of anaerobic digestion plants present in Pakistan. Manufacturing will be made by raising the open tender, which is in a process.

- Final specifications for material of construction for this digestor are being established to make an open tender.

In enzyme research, Professor Tabassum worked with Dr. Charles Lee from USDA-Albany to obtain specifically-synthesized DNA primers; they worked together to confirm sequencing and synthesis of genes important to code for specific enzymes that will be used in this research to convert biomass to bioenergy.

- The following primers were created in order to clone a protease gene from thermophilic and mesophilic Bacillus strains.

dpnpr1F 5'-ScDgcVgtDgaYgcHcaYKM-3'
dpnpr2F 5'-caYgaRWtRacVcaYgcBgt-3'
dpnpr2R 5'-acVgcRtgBgtYaWYtcRtg-3'

dpnpr3R 5'-aDYaRRtaMgcBKStttRtt-3'
dpspr1F 5'-gtNgcKgtHHtNgaYaSVgg-3'
dpspr2F 5'-caYggHcaNcaYgtNgcHgg-3'
dpspr4R 5'-ggNSHHgccatNgaHgtDcc-3'
dp, degenerate primer. The sequences of degenerate primers are labeled by the degenerate DNA

In biomass research, Professor Tabassum's group initiated studies on using corn stover (the remaining stalks after harvest) as substrate but found some difficulties in grinding the stover to make a powder suitable for digestion studies.

- Utilizing lawn grass, pretreated with alkali treatment – autoclaved and without pretreatment – has proven more fruitful.

The USDA team bought, assembled, tested and began utilizing a set of fermentors that have been established to test the protocols established in this project. To date, research is ongoing in the following areas:

1. Characterization of solid waste biomass, including municipal solids waste (MSW), food wastes (including sub-par fruits and vegetables), ag-processing residues, grass clippings, tree prunings, paper wastes such as office waste, newspapers and packaging. Research on almond hulls and almond shells are particularly effective. The USDA has collected wastes from throughout the state of California to compare results for different almond varieties. This will make results more universal and potentially applicable to Pakistan. Almond wastes are a big biomass source in California, and also in Pakistan (a top-10 world-wide producer). Considering that almonds are a common biomass source for both the U.S. and Pakistan it is noteworthy that fermentation of almond shells and hulls is proving to be viable.
2. Analysis of pre-treated MSW as biomass feed stock continues.
3. Fermentation of easily-derived sugars from these wastes for ethanol production was already carried out for sugars obtained from almond hulls and was initiated for sugars from carbons derived from solid waste (MSW).
4. A series of fermentors were established, according to the project plan, for anaerobic digestion to produce methane, with establishment of colonies that will convert solid waste to biogas
5. The biogas from these experiments has been characterized; one interesting result is the variation in biogas composition as a function of inputs. .

The aim of our first year was to evaluate different chemical methods for the pretreatment of lignocelluosic feed stocks to remove the lignin which hinder the degradation of this substrate during ethanol and anaerobic digestion. The work has been started on all the milestones that has been mentioned in project. Thus we treated different substrate through different method to find out the efficient chemical treatment method for lignin removal.

1. Selection of waste substrates

The selected agriculture material for pretreatment were of agro industrial wastes were cotton stalk, cotton lint, wheat straw, bagasse, lawn grasses, corn stover, paper waste, almonds shells, Grasses grown on saline lands kallar grass, para grass and atriplex fruits and vegetable wastes. All the agricultural wastes were crushed to

Effect of different pretreatments and substrate concentrations and substrate of methane production.

2. Physical pre-treatment

The lignocellulosic wastes were shredded to small-size particles and then crushed by a grinding miller to powder form in order to increase its susceptibility to enzyme and bacterial attack. For this purpose, Agricultural waste material was grind and this grinding miller was carried out in NIBGE Fsd. Model AM 80 MX2, rotating at 50 Hz, equipped with a sieve with an equivalent pore size of 0.5- 20 mm and dried at 100°C overnight.

Vegetables and fruits wastes were treated separately by crushing and grinding. These materials was used without prior acid pretreatments.

Methods of chemical pretreatment

The selected waste material was treated with different chemical pretreatment methods.

Different strategies were used

i) Different concentrations of alkali NaOH for pretreatment of wastes.

ii) Alkali NaOH treatment and autoclaving at 121 ˚C for 10 min

iii) Autoclaving at 121 ˚C for 10 min with aqueous ethanol and alkali pretreatment method .

iv) Dilute acid pretreatment of lignooellulosic biomass.

Support Personnel: In Pakistan, posts were advertised for four Research Associates, three Field Assistants. Present positions include, one Research Associate and two Lab Assistants are already present. Other recruitments for Research associate are under process. For recruitment of research associate and field assistant, advertisement was made national and on NIBGE web. Three research associates and two field assistants has been selected. One research associate and two field assistants has been joined on 21-6-2012. Application of the Ph D researcher is in the process of registration to get admission in PIEAS, Islamabad to conduct the research on the topic related to the main theme of project. Other two research associate will get admission after joining.

Educational Impacts: Please provide information on the numbers of students and other junior collaborators (graduate and undergraduate students, healthcare workers, laboratory technicians, data collectors, etc.) involved in the projects, and discuss new courses or degree programs created (if any) or changes to existing course curricula as a result of your project.

In Pakistan, Four Research Associates, Three Field Assistants. One Research Associate and Two Lab Assistants are already present. Other recruitments for Research associate are under process.

Infrastructure Development: Please list any equipment acquired during this reporting period with grant funds and discuss the impact the new equipment will have on research and educational activities.

Professor Tabassum is actively purchasing several items that are critical for the success of this joint project, as listed in the proposal. The most significant is design and installation of a $50M^3$ anaerobic digestor.

Pakistan: Purchase of following equipment is under process (supply orders issued to the respective suppliers):

i. Biogas analyzer,
ii. French press,
iii. Nitrogen generator,
iv. Double water distillation,
v. Ice making machine,
vi. Water purification system,
vii. Gradient PCR machine,
viii. Shaking incubator,
ix. -86 Freezer,
x. Bench top centrifuge machine,
xi. 50 M^3 Anaerobic digester

All equipment definitely will strengthen the research capabilities at NIBGE.

The USDA team bought, assembled, tested and began utilizing a set of fermentors that have been established to test the protocols established in this project.

Publications: Please provide citations for any papers published or conference presentations made as a result of your project.

William Orts and Kevin Holtman made at least 5 presentations in which results and impact of this collaborative project was presented, including the following:

- Invited keynote at the 20th Annual Meeting of the BioEnvironmental Polymer Society, Denton, TX, September 2012
- Invited presentation at the 3rd Biobased Chemical Conf., San Francisco, August, 2012
- Invited panelist at the 1st Annual Anaerobic Digestion Conference and Expo, San Francisco, CA May 2012

Additional Funding: Please list any additional funding applied for or received to help support your project during this reporting period.

Industrial funding of $20,000 US from a company, Global Organic Energy, has been proposed and will likely be received in November.

Linkages with Government or Private Industry: Please describe any linkages developed with government agencies or companies interested in implementing the results of your project.

The USDA lab continues their interactions with the Salinas Valley Solid Waste Authority who are appraised of the results of this research and continue to rely on the data generated through this project to scale-up their digestor systems. Specifically, the co-PI, Holtman and his team on this research, is also installing a scaled-up digestor system in Salinas that expands this joint effort. More recently, this research has been joined by an industrial partner, Global Organic Energy (GOE), who collaborates on bioenergy and is interested in this research.

Interloop Mills Limited, Faisalabad, A private industry showed interest for development of bioculture for methane production from kitchen wastes in the pilot plant digester.

Problems Encountered: Please provide information on any problems you may have encountered in making progress on your project objectives and describe steps you are taking to resolve the problems.

There was delay in release of funds which slow down the project speed and ultimately effected the procurement of Equipment. The prices of $ has been increased the prices of equipment and consumables are increased two times from the previous estimates mentioned in project.

• There was cut on stipend for Research associate and Lab assistant. P.I of Pakistani side faced problem for recruitment. A request was made to HEC Pakistani side to increase. Stipend but HEC has denied the request.

Several difficulties have arisen within the USDA travel/visa office; specifically the visa for Miss Tanveer Majeed has been delayed several times. This problem occurred, in part, because the local personal dealing with the application did not fully understand the process and did not complete everything required during initial submission. As a result, the visa application was “dormant” for weeks/months. This situation appears to have been resolved, and Miss Majeed’s arrival is expected during November 2012. In Pakistan, funds are limited for procurement, with some issues in procurement of instruments especially in the biogas plant construction and fabrication.

Plans for Activities During the Coming Year: Please provide details on project activities during the next year, including planned exchange visits, training events, and ongoing research efforts.

The most significant activity will be the arrival of Miss Tanveer Majeed, a visiting student from Pakistan to the USDA-Albany labs for a 6 month collaborative research project, likely in November; as soon as the travel visa is obtained through the USDA.

Specific targets:

1) Optimizing enzymatic hydrolysis process.
2) Fermentation of pretreated wastes for production of ethanol by using recombinant yeast
3) synthesis of primers and sequencing for identification of methanogens and cloning of enzyme genes
4) PI of project will visit in January 2013 to William J. Orts, US Department of Agriculture, Albany, California, USA. The PI will spend several months to work on enzymatic treatment of biomass.
5) A Ph.D student, Tanveer Majeed has plan to work in Dr. Lee Lab in November 2012.

The analytical methods have been initiated but will be continued for the analysis of cellulose, lignin, hemicellulose, ash, total solids and volatile solids used for the characterization of waste substrates.

The work is progress for 1st year plan

1. Use of other chemicals for pretreatments of waste materials to make the process economically viable
2. Investigation of effective pretreatment methods for enzymatic hydrolysis and anaerobic digestion.
3. Cloning of genes enzyme involved biodegradation of substrates.
4. Installation and commissioning of $50M^3$ biogas plant at NIBGE

The USDA expects to begin publication of some research results.

Scale-up of the anaerobic processes continue in both Pakistan and in California, with both systems projected to be operational within the coming year.

Supplementary Information: If applicable, please attach copies of project-related workshop or conference agendas, course curricula developed, summaries of research data collected in the course of the project, or articles about the project appearing in newspapers, journals, or Web sites. **Please note that your report will be posted on the program Web site, so please do not include any data you do not wish to make publicly available at this point in your research.**

Indicators	**Reporting Period:**
1. Number of higher education partnerships between Pakistani and U.S. institutions (see note below)	1
2. Number of journal articles, technical reports, books, or book chapters (published or accepted for publication) resulting from your project during the reporting period	3
3. Number conference presentations resulting from your project during the reporting period	5
4. Number of training events (courses, workshops, seminars,	1

conferences, stakeholders' meetings) conducted on your project during the reporting period	
5. Total number of Pakistanis making exchange visits on your project during the reporting period	0* * Obtaining visitors' visas have been delayed
Number of women	0
Number of men	0
6. Total number of Americans making exchange visits on your project during the reporting period	0
Number of women	0
Number of men	0
7. Total number of exchange visits overall during the reporting period	0
8. Total number of Pakistani PhD students involved in the project	3
Number of women	1
Number of men	2
9. Total number of American PhD students involved in the project	1
Number of women	0
Number of men	1
10. Total number of all other Pakistanis not listed above who participated in your project during the reporting period (Include in this total those who were involved as researchers, MS or undergraduate students, technicians, or data collectors, as well as those who received formal training in workshops or courses or participated in conferences or stakeholders' meetings organized as part of the project.)	25
Number of women	5
Number of men	20
11. Total number of all other Americans not listed above who participated in your project during the reporting period (Include in this total those who were involved as researchers, MS or undergraduate students, technicians, or data collectors, as well as those who received formal training in workshops or courses or participated in conferences or stakeholders' meetings organized as part of the project.)	4
Number of women	3
Number of men	1

Note on Question 1: For the number of higher education partnerships, please count the partnership between your institution and your Pakistani counterpart's institution as one. If your project also involves collaboration with other Pakistani institutions / US institutions (universities, research institutes, government agencies, or non-governmental organizations), please add each such additional institution to your total.