Energeia Home

CAER -- University of Kentucky, Center for Applied Energy Research Vol.6, No.6, 1995

ENERGEIA

The Role of Activated Carbon in Tissue Culture Medium

S.C. Van Winkle and G.S. Pullman
Institute for Paper Science and Technology

INTRODUCTION AND BACKGROUND

Tissue culture is an in-vitro technique for replicating a plant without introducing the degree of genetic variability inherent in sexual reproduction. Tissue culture has proven commercially important for the replication of ornamental varieties of house plants and is used extensively in research for applications in forestry. Activated carbon (AC) was initially added to tissue culture media in an attempt to simulate soil conditions: today it is routinely included in many tissue culture media formulations. The benefits of using activated carbon often include greater plant survival, greater plant growth, and improved plant quality and vigor. In some conifer tissue culture systems such as Douglas-fir, benefits include higher culture initiation rates and improved plantlet survival and vigor.

In our laboratories at the Institute of Paper Science and Technology (IPST), AC is most often used in tissue culture systems where the objective is to start an embryogenic culture (somatic embryos) from naturally occurring (zygotic) embryos. (The zygotic embryo may be isolated by carefully dissecting a seed, typically a conifer in our case.) The process of inducing the first somatic embryo from a zygotic embryo is referred to as ''induction'' or ''initiation.'' Initiation is typically performed in small (15 mL) Petri plates on a semisolid medium which contains a full complement of nutrients and hormones. Following a successful initiation, a mass of early-stage somatic embryos may grow. These are physically separated from the rest of the tissue and transferred to a ''multiplication'' medium where embryos continue to replicate forming large numbers of genetically identical embryos.

The multiplication phase is performed within flasks of liquid media which enhances the speed of embryo multiplication, often resulting in thousands of early-stage embryos within a few weeks. The early-stage embryos may then be transferred to a development medium where they grow and develop morphological features similar to the immature zygotic embryo. The somatic embryos are then transferred to the cotyledonary embryo development medium. Following this stage, the somatic embryos bear a strong resemblance to nearly mature zygotic embryos. The final stage is germination during which the somatic embryos develop into plantlets.

Table I. Picea Abies, IPST Medium (3)

Basal Salts	Concentration (mg/L)
KCl	372.5
KNO_3	50
KH_2PO_4	85
$MgSO_4.7H_2O$	160
$CaCl_2.6H_2O$	220
KI	0.415
H_3BO_3	3.1
$MnSO_4.H_2O$	8.45
$ZnSO_4.7H_2O$	4.3
$NaMoO_4.2H_2O$	0.125
$CuSO_4.5H_2O$	0.0125
$CoCl_2.6H_2O$	0.0125
$FeSO_4.7H_2O$	13.9
Na_2EDTA	18.65
Organic Additives	
Sucrose	10,000
myo-Inositol	50
Casamino acids	500
L-Glutamine	750
Thiamine.HCl	0.05
Pyridoxine.HCl	0.05
Nicotinic acid	0.25
L-Asparagine	50
AC (optional)	2500
pH	5.8

The media for tissue culture have been developed empirically over several decades. A typical tissue culture medium is an aqueous solution of macronutrients (salts which are present in hundreds of milligrams perliter), micronutrients (salts present in tens of milligrams or less per liter), a carbohydrate supply, amino acids, an osmoti-cant, and plant growth regulators or hormones. As already noted, the medium may also contain activated carbon (AC). A gelling agent, commonly agar or a bacterially produced polymer (phytagel), is often included to provide support to the culture. A typical basal medium (no hormones) composition is presented in Table I. In addition to these components, a growing culture will pro-

(continued, page 2)

Rosalind E. Franklin Who was She?

Written by Professor Michio Inagaki
(11th March 1983)
(Hokkaido University, Japan)
Translated by Katsuo Osafune

Submitted and Edited by Dr. Harry Marsh
Department of Inorganic Chemistry
University of Alicante, Spain
03080 ALICANTE

INTRODUCTION

My purpose in writing this short article is to keep alive, not just the memory of Rosalind Franklin, but the appreciation of her contributions to carbon and coal science. She laid down the concepts of structures in coals within the rank range that we still use. At the same time, she commanded much public sympathy over the way she was treated with regard to the double-helix story. When I stumbled across a letter written some years ago by Michio Inagaki, I felt there was enough need and interest to introduce it to a wider audience.

(continued, page 3)

Activated Carbon, (continued)

duce unidentified substances which are released into the medium. Some of these substances are believed to be phenolic derivatives which inhibit growth. Others may be gas phase (CO_2 and ethylene) inhibitors. Evidence for a "critical mass" effect suggests that the developing culture also produces substances which promote growth.

Several hypotheses regarding the beneficial effect of activated carbon in tissue culture medium have been advanced. These include: sorption of medium components (organic and inorganic); sorption of agar impurities; sorption of inhibitory growth by-products; release of stimulatory substances; media darkening; pH effects; and catalyzed breakdown of sucrose during sterilization with subsequent sorption of hydroxy methyl furfural. Interactions are likely and could be more important than an isolated effect.

Results from initiation experiments at IPST indicate that different activated carbons may produce different success rates. These differences in performance are attributed to physical or chemical AC characteristics.

The goal of this study is to correlate initiation success with one or more physical or chemical characteristics of activated carbon. The primary benefits will be a better understanding of both initiation and the activated carbons which promote initiation.

Experimental

The experimental plan was conceived in three phases: characterization of AC, quantification of AC effects on initiation medium composition (sorption performance and/or release of compounds), and development of a suitable initiation system (bioassay model) to assess different activated carbons.

Based on prior results, two types of carbon (Sigma) have been chosen for initial study. These are marketed as acid-washed, cellculture tested, and non-acid washed (untested) activated carbons. The AC characteristics which will be examined include: porosity (nitrogen sorption, primarily); oxygen functionality (FTIR, Boehm titration); surface charge (electrophoretic measures); bulk charge (mass titration estimation of point of zero charge, PZC); and ash content/composition.

The performance of AC will be monitored through analysis of the liquid phase of the medium. Liquid chromatography (HPLC with radio-labeled compounds) will be used for analysis of organics and atomic emission spectroscopy (ICP-AES) will be relied on for the salts.

Though Douglas-fir and loblolly pine are commercially more important, Norway spruce was chosen for the bioassay model: Norway spruce cultures are initiated from mature seed, allowing experiments to be performed throughout the year; whereas, Douglas-fir and loblolly pine require immature seeds which are only viable for initiation a few weeks per year. A liquid medium is being developed for the bioassay model to facilitate chemical analysis and avoid difficulties posed by gelled media.

Results

Initial results from the characterization of the activated carbons are depicted in Figures 1 and 2. The activated carbon type which was used for tissue culture at IPST is designated as TCAC ("tissue culture activated carbon"). Two different lots are represented, designated TCAC1 and TCAC2. The carbon type for which unsuccessful initiation has been previously observed is designated NAC. Again, two different lots are represented, designated NAC1 and NAC2. The TCAC material is sold as acid-washed (HCI) whereas the NAC material has not been acid-washed.

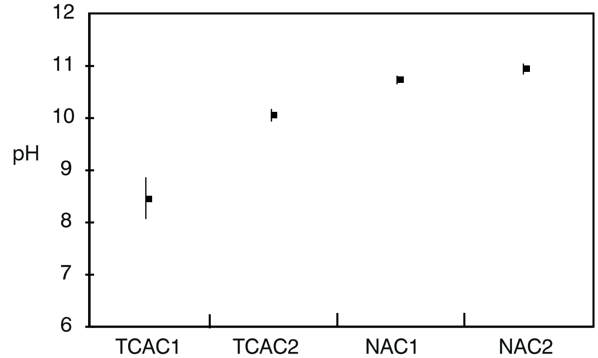

Figure 1. *PZC approximation, four different carbons (Tick marks designate mean values, bars represent standard error, based on five samples*

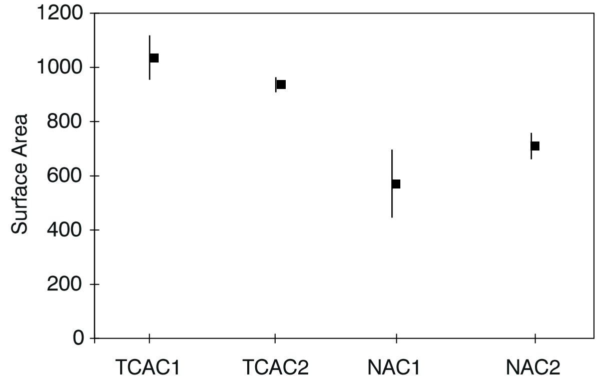

Figure 2. *Apparent surface area, four carbons. (Nitrogen/BET)*

Table II. Norway Spruce Bioassay Development				
	Observed Initiation (%)			
	Rep 1	Rep 2	Rep 3	Mean
10 NAA TCAC1	70	60	40	56.7
10 NAA TCAC2	12.5	40	50	34.2

- *Numbers in sample ID refer to amount of AC (mg/L).*
- *NAA designates naphthalene acetic acid.*
- *'TCAC' designates cell-culture tested AC; batches designated '1' and '2'.*

Figure 1 depicts results from the PZC approximation. From Figure 1, it may be seen that all of the activated carbons have a basic character. The non-acid-washed materials (NAC1 and NAC2) are more basic than TCAC1 or TCAC2. Note the amount of variability between the TCAC lots. Also note that the measurements tend to become less repeatable as the carbons become more neutral.

The surface area data in Figure 2 were produced using single point N_2 BET measurement. Again it appears that the four carbons are different. They all appear to have high surface area, consistent with microporous carbons. The surface area difference between carbon types is greater than the difference within a carbon type, with the tissue-culture carbons displaying somewhat higher surface area than the non-acid-washed carbons. Future work will focus on microporosity.

Initiation rates (%) from one experiment in the Norway spruce bioassay development are presented in Table II. The media for the two treatments contained 10 mg/L of the different batches of the same type of tissue culture activated carbon but were otherwise identical. Each treatment was replicated three times. While the means differed greatly (56.7% and 34.2%), the difference was not statistically significant at P=0.10 or less for these two batches of the same AC product. Currently, it is uncertain whether activated carbon significantly increases the variability in successful initiation. The variability shown in the tissue culture

(continued, page 3)

Activated Carbon, (continued)

system presents us with a challenge for correlating the physical characteristics and biological effects of AC.

Initial Conclusions

The four activated carbons examined appear to be significantly different with regard to the parameters tested (apparent surface area and bulkcharge). Other data suggest that they also differ in ash content and composition. The level of batch-to-batch variability for the same type of product was unexpected. Characterization of these and other activated carbons is ongoing.

A tissue culture bioassay employing a liquid medium has been developed which responds to differing levels and types of AC. The liquid medium will be the basis for comparing AC differences in sorption performance. Future refinements will enhance the sensitivity of this assay.

It is expected that this research will shed light into the relationships between AC physical characteristics and sorption performance. While the particular emphasis of this research is on the use of AC in plant tissue culture systems, information obtained will likely be of value in other areas of AC usage.

Steve Van Winkle completed his M.S. degree at Virginia Tech in Wood Chemistry. Currently he is working towards his Ph.D. at IPST.

Rosalind Franklin, (continued)

In 1981, Professor Michio Inagaki published, in Tanso, No. 106, pages 130-131, an article describing the personal memories of his experiences when working with Rosalind Franklin in France. I feel that the following article, written by Professor Inagaki, adds significantly to the debate of her life. When working in the University of Newcastle upon Tyne, U.K. I had with me Katsuo Osafune, a student from Japan, and together we produced this translation of the article. Sadly, Osafune passed away some years ago. I have consulted with Professor Inagaki and I have his permission to submit this translation of his original article to ENERGEIA.

As a scientist working in the area of carbon materials I have always been immensely impressed with the contributions that Rosalind Franklin made in her short life (1920-1958). When teaching the basics of structures in carbons I always refer students to her publications in Proceedings of the Royal Society of London 1951, A209, 196, "Crystallite growth in graphitizing and non-graphitizing carbons," and in Acta Crystallographic, 1951, 4, 253, "Structure of graphitic carbons."

There is no doubt that her life and experiences as a researcher were tumultuous, to say the least. In a way, her experiences as a researcher are an accurate description of attitudes to female researchers at that time. It has been known for a professor not to teach a class because a female was present in the class. How times have changed. Although the debate over her contributions to scientific understanding of structure in both inorganic and biochemistry will continue, make no mistake, the world is now a very different and better place for the female researcher, particularly in England, where the experiences of Rosalind Franklin became generally known.

*However, there is an ironic twist to the tale. Rosalind Franklin was researching in a period of time when interest and understanding of the parallel processes of inorganic and biochemical evolution were expanding extremely rapidly. Coal, a material she studied, is an interesting material originating from a botanically derived material, biomass (biochemical evolution) but created by the coalification process (inorganic evolution). In the fall meeting of the American Chemical Society, Fuel Chemistry, Chicago, 1993, 38(4), 1270-1274, P.G. Hatcher, K.A. Wenzel and J.L. Faulon published a description of reactions occurring within wood during the early coalification period, with a view to elucidating the structure of vitrinite. During peatification of woody tissue, cellulose is biodegraded first, leaving the lignin structures 'unsupported.' In lignites, this lignin adopts a **"helical structure."** With increasing coal rank, it is thought that the original helical structure of the lignite degenerates and is almost lost in the bituminous coals. If Rosalind Franklin had lived would she have applied her instinctive intense intellectual capacity, now experienced with helical structures, to coals? Whatever we may think, it is in some way appropriate to associate with her the helical nature of structure in the two materials which received her attention, DNA and coal.*

And, what a pity that she had to suffer so much. I personally met her only once, in University College, London in 1952, and I remember her as a sad and lonely figure in the middle of a reception for one of the first Conferences of the Society of Chemical Industry on Industrial Carbon and Graphites.

However, there is more. I made several attempts when in The University of Newcastle upon Tyne, to establish a Named Lecture of a Professional Society to perpetuate the name of Rosalind Franklin. So far, I have failed. Now is a good time to make another attempt. I appeal to the coal and carbon communities in general, and to individuals in particular, to write to me in my Alicante address giving me specific proposals of ways to establish some form of memorial.

Upon hearing the name "Rosalind Franklin," most readers will probably say, "who was she?". However, many will recognize her name by "the Franklin Model" or "the P-value of Franklin." Franklin was a promising scientist during the first half of this century. A book, entitled **Rosalind Franklin and DNA, A Stolen Glory,** was written by Ann Sayer in 1979. The book describes the personal character and history of Rosalind Franklin in some detail and corrects Watson's misleading description of Rosalind, which appeared in "The Double Helix". In 1962, Watson and Crick received the Nobel Prize with their colleague, Wilkins, for their important contribution to the establishment of the double-helical structure of deoxyubonucleic acid (DNA). In 1968, Watson wrote "The Double helix," which he regarded as a "personal memorandum of the discovery of the double-helical structure of DNA." However, Sayer insisted in her book that Watson also incorrectly described her as a rustic, obstinate, aggressive, arrogant woman. She expressed indignation against the decision of the Nobel Prize Committee because it ignored the important

(continued, page 4)

Rosalind Franklin, (continued)

essential contributions of Franklin to the double helix work. Franklin had provided the important data from X-ray diffractions for the structural analysis of DNA. Portugal and Cohen criticized Sayer's book in that her advocacy for Franklin was too emotional.

Rosalind Franklin was born in London on the 25th of July, 1920 as the second child of Jewish parents. She was brought up alongside her brothers, and had no inferiority complex with regard to males. In 1938, she entered the Newham College of Cambridge University. After graduation she worked as an assistant to Professor R.G.W. Norish. While working with him, she became a friend of Adrienne Weil, who had escaped from France to avoid the first world war. Rosalind received many invaluable suggestions, as a female researcher, from Weil with regard to the French crystallographers with interest in carbon materials. After leaving Cambridge she joined the laboratories of the British Coal Utilization Research Association in Leatherhead, U.K. where she studied the microstructure of coal. In 1945 she received a Ph.D. from Cambridge University. In the autumn of 1946 she met Dr. Marcel Mathieu, with whom she maintained a friendship for many years. Mathieu was fond of her as if he was her father and Rosalind respected him. Dr. Mathieu was interested in the catalytic growth process of carbon and the graphitization process. (It is probably from this connection that she developed a research interest in structure within carbons in general). So her French connection was established. In February of 1947 she went to Paris to join the research staff of CNRS. She studied carbon structure using X-ray diffraction. Dr. J. Méring taught her the techniques of X-ray diffractometry.

Dr. Michel Oberlin (now deceased) described Rosalind as she was at CNRS in a private letter to the author (Inagaki) as follows:-

"I joined CNRS in 1948, and met Rosalind there. She could speak French almost perfectly, except for a very slight English accent. In those days, Miss Agnes Mathieus-Sicand (later, Dr. Agnes Oberlin) also studied under Mathieu, using electron microscopy. We used to go dancing with Rosalind travelling to the dance as a pillion passenger on our motorcycles. She also enjoyed riding on a motorcycle alone in Paris. We liked swimming and we used to go to the pool near CNRS during lunch. When she was relaxing, she was a sound and cheerful young lady. Mr. J. Maire joined us in 1949, and began a study on graphite acid under her direction. One day when she came out from the laboratory, her cheeks were dirty. On pointing this out, she replied, 'This is not dust, just graphite.'

"We used to brew coffee in an Ehrenmeyer flask. Coffee was rationed in Paris at that time. She had many discussions with Professor Jacques Mering (who worked with Maire on the graphitization process) in the "coffee-room." In 1950 and 1951, based on these discussions, several papers were published in the journals of ***Acta Crystallographica and Proceedings of the Royal Society.*** *Most of the researchers met in the "coffee-room" for discussions. The atmosphere was completely free and active. She enjoyed her stay in Paris very much and it was perhaps the happiest time of her life. After returning to Britain, she returned from time to time to Paris and the French Alps."*

In early 1951, Franklin returned to London. "From a personal point of view, nobody might appreciate her for her decision. However, she wanted to test herself by challenging the new field of crystallography, i.e. its application to biology". The saga on DNA is described in detail in three books. But, the book written by Portugal and Cohen is probably the best because of its objectivity. In the book, the death of Franklin is described as follows:

"In September 1956, she was taken ill and was sent to the hospital. On the 16th of April 1958, she died after one and a half years' struggle against cancer. "

We know Franklin's work well from her excellent studies of the X-ray diffraction characteristics of carbon, which were completed and written during the four years of her study in Paris. Dr. S. Sanada visited her after establishing the double-helical structure and a report of this visit is to be found in "Tanso". She was a distinguished researcher in the science history of DNA and tobacco mosaic virus. In conclusion, her life struggle suggests to me the early existence of sexual discrimination among some scientific researchers. Finally, her life also suggests to us the difficulties in the evaluation of one's own achievements.

For more information on Rosilind Franklin, please contact the author.

1995 International Ash Utilization Symposium -- Great Success

The International Ash Utilization Symposium was held October 23-25, 1995 with 293 participants from industry, academia, and government attending. The Keynote address was by Richard Kruger of Ash Resources (PTY) Ltd., Randburg, Republic of South Africa. There was also an In Memoriam Address honoring the late Barton A. Thomas presented by Charles J. McCormick, Advanced Pozzolan Technologies, Inc., Atlanta, GA as well as the announcement of the Barton A. Thomas Best Presentation Award sponsored by Mrs. Virginia Thomas. It was a highly successful international meeting with speakers from 13 countries. Another meeting is planned for October 20-22, 1997.

COMMENTARY

a la moda

Frank Derbyshire, Director, CAER

Charles Dickens took some pains to describe the evils of the workplace, including the office which, if it has not always been a location for white collar servitude, has certainly held a tradition of formality in dress and behavior. The dress code, whether officially ordained or merely implied, has tended to be more rigorous for men than for women. One could speculate that this is a reflection of stuffy male attitudes, caution, or lack of imagination, rather than deliberate discrimination. Employers have also, in general, encouraged uniformity in dress and behavior for the reason that, as is desired in the military, it suppresses individual expression and imagination, discourages insubordination, and helps to maintain authority. However, such a policy is not at all conducive to creativity, or to modern methods of management, which owe rather less to the tactics of Genghis Khan than to the concept of leading others in a collective and interactive process of problem definition, planning, and execution (not the sort of execution that was so favored by The Great Khan - although some of his measures still retain a certain vestigial appeal). Along these lines, a trend that is developing across the Western world is to loosen these long-established and rather rigid codes. In many organizations, the wearing of a tie is becoming optional, and Fridays are often given to a "dress down" day (still following the thinking of The Mighty Khan, there might be some justification for inaugurating a "dressing down" day), when employees of both sexes appear in casual dress - a term that is subject to individual interpretation, and whose manifestation may range from the elegantly nonchalant to the sartorially ludicrous.

Contrasting conference fashions a la moda internacional

From left to right: the author; Professor Isao Mochida, Kyushu University, Japan; Professor Roy Jackson, Monash University, Australia, at the International Conference on Coal Science, Oviedo, Asturias, Spain, 10-15 September, 1995; published in ***La Nueva España, Oviedo, Jueves, 14 de Septiembre de 1995.***

Descriptions as published in La Nueva España: from left to right: "zapatos náuticos, aire informal"; "...visten impecablemente, según el modelo del ejecutivo estándar..."; and "...acude de pantalón corto a algunas sesiones".

Translation from left to right: "boat shoes, informal attitude"; "...impeccable dress, the model of the standard executive..."; and "...attends some sessions in short pants".

While the prospect of dressing unconventionally for work can be attractive, it requires confidence in the belief that one's colleagues will behave in like manner. Like the adolescents we (some of us) once were, we try to express our independence by appearing to be radically different - provided, of course, that all of our contemporaries do the same. The designation of a specific day of the week for the donning of more adventurous modes of attire provides the confidence that everyone else will also arrive in a creative little outfit - or will they? In my own experience, a common dilemma when preparing to attend a conference is to determine how the others will be outfitted. Some meetings, usually those held in warm weather, deliberately encourage informal dress - but not all of them. Whichever the situation, at least one person will get it wrong. In a crowd of conferees that could be understandably confused as a collection of beach bums, there will be someone perspiring unhappily within the confines of regulation issue dress. Conversely some lone, coolly clothed dude, adrift in a sea of suits, will be yearning for the comparative comfort of a collar.

The extent of relaxation of the normal dress code also depends upon the type of office environment. In a university, faculty tend to dress to their own comfort, sometimes with more than a hint of eccentricity. Even then, if important visits are to be made, or important visitors are to be received, the academic will usually rise to the occasion, and resurrect long-dormant, and ill-fitting garments whose cut and texture belong to a fomer era and a slimmer owner. However, in certain sectors of commerce and industry, and in the legal and medical professions, where there is a need to inspire the confidence of one's clients, there is a limit to the informality that can be achieved. Not many patients, for example, have a sense of humor that is sufficiently advanced to be able to appreciate their doctor's diagnosis, if he or she is sporting a luminous bow tie or similar attire. On the other hand, modern expectations of a doctor's bedside manner are no longer consistent with a morning coat, starched collar, and starched attitude.

Most office workers struggle under self-induced pressures and deadlines, whose effects are exacerbated by electronic implements of torture - the computer, the fax, e-mail, et cetera. Any measures that help to alleviate these tensions can only improve the quality of the work climate, and will probably augment productivity. It must also be recognized that not everyone can accept the adoption of a more worker-friendly appearance, especially those accustomed to years of rigid routine, and imbued with the more restrictive attitudes

(continued, page 6)

a la moda, (continued)

of earlier generations. Some hold the belief that wearing a "uniform" provides a badge of rank and seniority. To them, clothing is a form of security blanket, providing a statement of the bearer's position in the hierarchy, and reducing the burden to establish a leadership role through example and personality. Consequently, the substitution of "civvies" for the "uniform" can be worrisome, and bring feelings of insecurity and loss of status. At the opposite end of the spectrum, alternate wardrobes should be approached with moderation. Excessively enthusiastic participants may require words of advice and caution upon the subtle distinctions between the assumption of discreetly tailored apparel and "putting on the dog." Although the colorful debut of a colleague clad in carnival raiment could evoke comic relief, most experts would maintain that such undue license in costume selection would have an overall disruptive effect. There may be readers (if they have managed to persist to this point) who would disagree with my assessment of this topic. Normally, I would be pleased to arrange a meeting to try to resolve any differences, but in this case, I'm not dressed for the occasion.

Rosalind Franklin, (continued)

Energeia is published six times a year by the University of Kentucky's Center for Applied Energy Research (CAER). The publication features aspects of energy resource development and environmentally related topics. Subscriptions are free and may be requested as follows: Marybeth McAlister, Editor of **Energeia,** CAER, 3572 Iron Works Pike, University of Kentucky, Lexington, KY 40511-8433, (606) 257-0224, FAX: 606-257-0220,

UNIVERSITY OF KENTUCKY

Center for Applied Energy Research
3572 Iron Works Pike
University of Kentucky
Lexington, Kentucky 40511-8433

Non-Profit Organization
U.S.Postage
PAID
Lexington, Kentucky
Permit No. 51