



# Seafood NET

# NEWS

LOUISIANA SEAFOOD RESOURCES

FALL 2001

## *New Look For Seafood Processor News*

### **Updates USDA Aquaculture Outlook 2001!**

**O**n October 10, The USDA Economic Research Service issued a report, entitled: "Aquaculture Outlook," Report No. LDP-AQS-14 informed the examination of the U.S. aquaculture industry: production, inventory, sales, prices, inputs, and trade of catfish, trout, tilapia, salmon, mollusks, crawfish, shrimp, ornamental fish and new species. The amount of seafood imported into the United States over the last 5 years has grown considerably. Much of the increase has come from aquacultural production in other countries specifically targeted to high-value markets, like the U.S.

Exporting countries can do this through the introduction of products that they have an economic advantage in producing or they can produce products that have an existing market in the U.S. Two examples are imports of crawfish meat from China and frozen catfish fillets from Vietnam. The domestic aquaculture industry is expected to face strong competition for the remainder of 2001 and into 2002 from imported aquacultural products. An electronic copy of the

full report is posted at:

<http://www.ers.usda.gov/publications/so/view.asp?f=livestock/ldp-aqs/>

### **New GAO s Reports Involved with Foodborne Illness Surveillance and Food Safety**

**O**n September 7, the US General Accounting Office Issued a Report, Titled: "Food Safety: CDC Is Working to Address Limitations in Several of Its Foodborne Disease Surveillance Systems," GAO Report Number 01-973, which is posted at <http://www.gao.gov/cgi-bin/getrpt?gao-01-973>.

On October 10, the US General Accounting Office Released a Statement that was presented that day by GAO's Robert A. Robinson, managing director, natural resources and environment issues, entitled: "Food Safety and Security: Fundamental Changes Needed to Ensure Safe Food," GAO Report Number 02-47T, before the Subcommittee on Oversight of Government Management, Restructuring, and the District of Columbia, Senate Committee on

Governmental Affairs, a copy of which is posted at: <http://www.gao.gov/cgi-bin/getrpt?GAO-02-47T>.

## What Is ATP Luminometry?

*Drs. Charles Hagedorn and Nancy Love, Virginia Tech*

**A**denosine-triphosphate (ATP) is the energy source common to all living organisms. "It disappears within two hours after cell death, and the amount per cell is generally constant" (1). Measurements of extracted cellular ATP can be directly related to the the number of bacteria present, and can be a very rapid method for determining microbial numbers and qualifying sanitation procedures.

The easiest way to measure the ATP level is through the use of the firefly light emitting mechanism. This mechanism utilizes the luciferin-luciferase reaction to emit light in the presence of ATP. Generally, one adds these enzymes to a sample from which the ATP has been extracted.

The light produced is apparent within almost seconds, and is proportional to the amount of extracted ATP present. Theis light emitted must be measured by a luminometer or scintillation spectrometer. The results of this measurement are returned in RLU's (Relative Light Units). A high value indicates a high number of microorganisms present, while a low number indicates few bacteria present.

### Luminator Procedures:

The procedures required for luminometry testing vary slightly depending upon several issues: there are many commercially available luminometers that basically all functions in the same manner, however sample volumes, threshold readings, time required and reagents necessary all vary slightly from manufacturer to manufacturer.

The basic procedure however is the same for all luminometers:

#### 1. Collect samples.

If your sample is not liquid form, it will be necessary to suspend in an appropriate broth or buffered solution.

**2. Extract ATP from the sample** using extractant, usually purchased from the luminometer manufacturer, in order to release the ATP from the cells. The ATP must be released into solution in order for it to be detected.

**3. Add reagents** (luciferin and luciferase extracts), also purchased from the manufacturer, as directed by the manufacturer. (Volume and sequence will vary from manufacturer to manufacturer)

**4. Mix**, and quickly measure light output in luminometer. The basic procedure is quite simple and very rapid, taking only a few moments once the sample is prepared. This immediate return of results is what make luminometry so appealing to industry and researchers alike. Previously, a minimum of 24 hours was required for any type of microbial number determination. Rapid test kits are also available for many common food/beverage items as well as surface and water sampling.

These rapid kits usually involve a swab held in a specialized container which also hold the reagents necessary for testing. The reagents however, are separated from the swab by a layer of material that is easily punctured to allow mixing of the reagents following sampling. On site, the swab is removed from the container, the surface or material is swabbed according to a predetermined protocol (which includes a defined area or volume) and then the swab is immersed into the reagents in the tube. Within a few seconds, the entire contraption is inserted into the luminometer, and the

RLU's are returned. There are many variations to this technique that can provide more information than a simple RLU reading that determines the overall microbial load. If the sample is not extracted prior to testing, the background level of ATP present can be measured.

This test is usually run along with an extracted sample in order to determine how much ATP is present that is non-microbial in nature. Failure to run this test along with your extracted sample prevents you from being certain that the RLU value obtained is due in entirety to the microbial population. You can also perform an enrichment test on samples by culturing your sample in broth for 18-26 hours prior to testing. While this procedure reduces the rapidity of the technology, it can provide useful additional information such as viability and testing accuracy.

### **Pros and Cons:**

The rapidity and simplicity of this procedure clearly demonstrate the appeal of luminometry, however, there are several drawbacks associated with the technology. The main issue of concern is that the numbers returned (RLU values) are not at all comparable to plate counts or MPN values. Before implementing this technology into an industry or lab, a validation procedure must be completed on any and all types of samples in order to make sense of the values returned. For example, an RLU value of 800 means nothing if you haven't validated the machine by comparing plate counts to RLU values. If you are typically using the same types of samples, (i.e., soil or plant material), you would first need to run luminometer tests along with plate counts in order to correlate the data so that in the future, an RLU value of 800 has a microbial load value associated with it.

The RLU value will change drastically according to sample material so, plate counts or some other means of obtaining microbial numbers must be

completed along with luminometer values until a correlation can be made for each material.

This is time consuming and expensive, but eventually, once the correlation has been made, luminometer testing is all that is necessary. As mentioned above, the RLU values change drastically according to sample material even if the microbial load is exactly the same. There are several reasons for this which also have to be considered when comparing RLU values.

One reason is that there is an inherent amount of ATP presents in most materials that are not microbial in nature. This must be taken into account. This data can be obtained by running the sample first without extractant, but this adds yet another step to the procedure. Another reason is that "quenching" of the light emitted are common, and inherent to the sample type. For instance, a dark, or thick sample (molasses or glue) with the same amount of bacteria present per volume as a sample such as water, may not have the same RLU value.

The dark/thick sample will not emit as much light, and therefore the reading will be lower. This causes problems in that one might get a low reading, however, the microbial load is actually high. This problem can be combated by diluting as well as completing quenching studies to determine the percent quenching value which you can multiply your reading by in order to adjust to the actual value.

Cost is another issue that must be considered. Purchasing the luminometer is a one time expense that is easily justified in the rapidity and ease of use. However, the test kits (reagents, extractant, etc.) and supplies needed must be purchased often. While they are not outrageously expensive in most cases, they are more expensive than petri dishes and agar used for plate counts. In industry however, timing is essential, and money is abundant. Academic labs and governmental agencies however, may not be able to justify the price on rapidity and convenience.

## Links to Other Sites on Luminometry:

### **Biotrace**

<http://www.biotrace.co.uk/index.cfm/application/frameset/product/menustyle=product> This is a commercial site offering luminometer supplies and equipment. This site explains the technology and concepts behind luminometry as well as directions for sampling and testing using their machines.

### **Turner Design**

[http://www.turnerdesigns.com/applications/998\\_2620.htm](http://www.turnerdesigns.com/applications/998_2620.htm) This is another commercial site that not only offers luminometer equipment and supplies, but a nice description of the technology and applications as well as information concerning calibration, validation and sensitivity.

## **Smoke From September 2001 SCP and HACCP Training Classes at LSU!**

**W**e had 18 participants in SCP class and 20 participants in HACCP class. Our participants were from Louisiana, Kentucky, Missouri, Texas, Mississippi, Minnesota, Florida, and Mexico. Sorry, for people who could not come due to flight cancellation. We hope that you could join us in other seafood training courses. Our instructors are from the State of Louisiana, Department of Health and Hospital, Mr. Mike Cormier; Mr. David LeRay from U.S. FDA, New Orleans; Dr. Bob Price from University of California, Davis; Dr. Michael W. Moody, Department Head, LSU Food Science; Dr. David Bankston, Engineer, LSU Sea Grant College Program, and Dr. Voranuch Suvanich, Interim Seafood Technologist, LSU Sea Grant College Program.

We have posted the pictures from both trainings on SeafoodNET at <http://www.lsuagcenter.com/seafood/activities.htm>

## **The Third Wire...**

*David Bankston*

**Y**ou have probably heard reference to “the third rail” as in “Social Security is the third rail of politics”. This refers to the belief that any politician who messes with Social Security has signed his death warrant for future elections. The third rail expression comes from the way that electric-powered subway trains get their power. These trains run on two rails, which are very, much like the rails used for regular trains.

The difference is that the subway has a third rail between the tracks, and the third rail is the *Ahot@* leg of the electric power distribution system with the regular rails being grounded. With this setup, if a person touched the third rail and ground at the same time, electrocution could result.

We don't have third rail systems or subways in Louisiana, the closest that we come are the street cars in New Orleans that get their power from an overhead electric line. But we do have a third wire in most of our electrical systems in homes and businesses. That third wire is the one that connects to that “extra” prong, on a 3-prong plug. While the third rail is a potential source of danger, the third wire is actually a safety feature. It is intended to be grounded through the electrical wiring.

To understand how this is a safety feature, we need to look at the rest of the wiring. In a 110/120-volt system two wires are necessary to deliver power. One is the neutral which is usually connected to a ground and the other is a hot wire in which the voltage cycles from being higher than ground to being lower than ground. The current, which is the flow of electricity, flows according to the voltage, changing or alternating direction as the voltage changes from a value above ground to a value below ground. (That is why it is known as AC or alternating current power.) When working properly, the current flows from the hot wire through the electrical device being powered and then to the

neutral. For example, it may be powering a motor in a electrical drill, which is electrically isolated from the case of the drill and the person using the drill. Since the electricity has only one path, which it can travel - through the motor to ground - the person using the drill does not receive any current.

However, as we all know, things can go wrong and the electrical isolation can break down. It may be from moisture or dirt bridging the air gap between the motor and the case, from insulation around the wires breaking down or from other causes. When this happens, the case of the drill can become electrically hot - it is connected to the hot wire.

Now the person using the drill is an alternate path for the electricity to flow. If it is a good enough path, enough current will flow through the person to ground to severely shock or electrocute the person. Seafood processing plants are often wet environments, which means that the path through the operator to ground is often quite good. Even if the current is not great enough to cause damage directly, it could cause injury because the person's reaction to being shocked could cause injury (slips and falls, getting caught in machinery etc.).

If everything is working properly, the two-wire setup is good. The problem arises when something goes wrong and the electrical isolation is broken down. Then, the third wire is needed. The third wire is connected to the electrically conductive case at one end and a ground at the other. This gives the current a much easier path to flow through to ground than the path through a person to ground. This means that very little, if any, current will flow through the person because it will take the easiest path to ground. The results are that for relatively minor breakdowns in the electrical isolation, current will flow to ground and the person will not be shocked.

If a serious breakdown in the electrical isolation occurs (perhaps a direct short between the hot wire and the case) the third wire will still protect the operator while allowing a large enough current flow to trip the circuit breaker.

This sounds good - even if something goes wrong with the electrical isolation (a wet industrial environment is conducive to such problems)- the third wire can prevent harm. There is a catch, however, as there frequently is. The catch is that in order for the third wire to provide protection, it must be intact and connected to a ground.

This is not always the case. Have you ever seen a 3-prong plug on an extension cord that had the bottom prong cut or broken off? Have you ever seen a 3-prong plug used with a 3-prong to 2-prong adapter without the wire pigtail (assuming it is there) being connected to a ground? Is that third prong in the 3-prong outlet even connected to a ground?

In all of these examples, the third wire is not connected to ground and thus cannot provide an alternate path to the operator for electricity to flow. If this happens, the third wire is no longer a safety feature; it is just useless. In addition to being potential safety hazards, poor or non-existent grounds can cause poor or erratic performance of electronic equipment such as computers. Now, some of you may be familiar with wiring in older buildings, which has only 2-prong outlets. Power tools were used with these outlets. That is true, however, the safe way of using a tool was to connect a separate wire from the metal case of the tool to a ground.

This provided the third wire. Although it may not have been commonly done, since most of the time the normal electrical isolation worked well, it was recommended by tool manufacturers and included as part of the operating instructions.

There are also many current tools with only a 2-prong plug. These do not provide a separate third wire but they feature "double-insulation"; that is they have two layers of electrical isolation between the power and the operator. This means that if one layer fails, the other can still provide protection. These tools usually feature a plastic (non-electrically conducting) case as part of the double-insulated feature.

**See You Again in Spring 2002!!**