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APPLICATIONS OF BIOCHEMILUMINESCENCE IN QUALITY ASSURANCE OF FOOD PRODUCTS

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Abstract

To attend the food industries demands, rapid and sensitive methods have been developed to evaluate microbiological quality of several foods and water. The cleanliness of the environment in which foods are processed and stored must be monitored. Newly refined testing methods allow rapid monitoring and detection of bacteria, pinpointing any sources of contamination in food handling, storing and processing. Bioluminescence is light produced by a chemical reaction within an organism. The ATP bioluminescence assay (test) is based on the fact that all living cells contain adenosine triphosphate (ATP). ATP bioluminescence is not a microbial count method but it is sensitive enough to detect the ATP content of individual cells in small numbers. ATP is determined by using its reaction with luciferin, a light-emitting molecule found in fireflies.

***Key words**: bioluminescence, ATP, luciferin, luciferase, HACCP*

Introduction

The food industry has the responsibility to produce safe and wholesome food and providing this assurance to consumers is the ultimate goal of food microbiologists.

In contrast to quality control (QC), which is a reactive system that focuses on legal requirements and emphasizes statistically relevant measurements, quality assurance (QA) is a preventive approach that emphasizes operational procedures. These procedures must be robust, regularly reviewed and focused on the consumer. To establish QA/QC parameters, the food microbiologist uses two approaches. The first sets out to determine the total load of microbes in a sample, and the second attempts to determine the presence or absence of a particular microbial species, usually a pathogen or related type used as their indicators. Thus, while the first type of microbiological quality assurance test aims to establish that food products meet statutory requirements, the second type of analysis is focused on public health impacts with regulatory requirements as an integral part of the testing procedure.

In addition to the general testing requirements under QC programs, there has been an added element of quality assurance that is being pursued vigorously under the implementation of quality management systems such as Hazard Analysis & Critical Control Points (HACCP) plans. To further enhance the utility of these systems, there is a need to develop rapid microbiological detection techniques that are sensitive and accurate. Accordingly, much effort has been devoted to shortening assay times and to replacing the visible endpoints with alternative measurements. Real time or near real time methods for monitoring microorganisms are essential for implementation of a HACCP program, determine contamination, or the quality of a food or beverage item. What is needed is a microbiological test that is able to analyze a batch of food non-destructively, on-line and with the required accuracy, sensitivity and specificity, so the new biotechniques are playing a key role in helping microbiologists to assure food safety and quality (Lappalainen, 2000).

Biotechnology plays a major role in ensuring that the food we eat is safe. It's critical that perishable foods like meat, milk and fruit juices be routinely screened for the presence of harmful bacteria that could cause illness. As well, the cleanliness of the environment in which foods are processed and stored must be monitored. Newly refined testing methods allow rapid monitoring and detection of bacteria, pinpointing any sources of contamination in food handling, storing and processing. These biotechniques include: electrical methods such as impedance/conductance; chemical methods such as direct epifluorescent filter technique (DEFT); bacterial ATP bioluminescence, flow cytometry, biosensors, and agglutination /immunological assays; nucleic acid technologies such as polymerase chain reaction (PCR), ribotyping and microarrays.

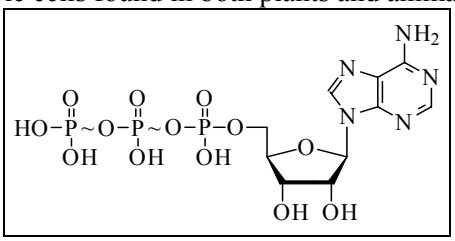
The ATP bioluminescence technique

A main application for ATP bioluminescence is quality assurance by surface testing in food processing plants to determine contamination of equipment and products (Griffiths, 1993, 1996; Poulis, 1993).

Some of the more commonly known bacteria which scientists are seeking to defeat include *E. coli*, *salmonella*, *staphylococcus* and *streptococcus*. These are among the bacteria which cause food poisoning. One of the quickest and most useful methods of finding and tracking the source of bacteria that can contaminate food like beef, pork and poultry is ATP bioluminescence (Orth, 1996). Bioluminescence is light produced by a chemical reaction within an organism, a phenomenon observed in many organisms such as fish, fungi, bacteria, worms and beetles. It has been

reported to occur in 17 phyla and at least 700 genera. Varied enzymes, collectively termed luciferases, and their corresponding substrates luciferins, mediate this biological production of light. In addition, other required specific co-factors and buffers are unique to each system.

ATP is the abbreviation for adenosine triphosphate, a key biomolecule in all metabolically viable cells found in both plants and animals:



The ATP bioluminescence assay is based on the fact that all living cells contain the molecule adenosine triphosphate. ATP bioluminescence is not a microbial count method but it is sensitive enough to detect the ATP content of individual cells in small numbers (Nilsson, 1984). ATP provides the energy required for cellular functions. The concentration of ATP is directly related to the number of bacteria cells present in a sample. ATP is a chemical measurement only and does not measure the number of bacteria. Total ATP does not correlate to the number of live microbial cells (bacteria, fungi, algae, etc.). For example, algae contain much higher levels of ATP than bacteria. Even a small, near-invisible clump of algal cells, or organic film deposit, contains high levels of ATP. If pure cultures of bacteria are used and if they are assayed in the same metabolic state, a close correlation with the number of cells is possible. However, since ATP is so very sensitive, real world samples and real world microbial cultures yield total ATP values which do not correlate with the number of cells.

There is interest in developing ATP bioluminescence for the detection of specific microorganisms. Current research is focused on combining ATP bioluminescence with techniques such as immunomagnetic separation for specifically removing target organisms from food, or by specifically lysing target cells with bacteriophage.

The problem with most foods is that they also contain ATP from non-microbial sources, and that the ATP content of microbial cells is variable depending upon their nature, the type of microorganism (e.g., bacterium, yeast) and their physiological state. In addition, the assay has no specificity. ATP is also present in food products, for example, in the muscle cells of meat. Therefore, to accurately determine bacteria levels on something like a beef carcass, the ATP contained in the food itself (background ATP) must

first be removed. Removing background ATP in samples of products such as raw milk, beef and poultry takes about 10 to 15 minutes. Then the microbial levels can be determined. Even with this extra step, the test is much swifter than conventional microbial testing, which takes 24 to 48 hours (Gracias, 2004). Because ATP bioluminescence can indicate the presence of food residues, it is also particularly useful for rapidly determining if the food preparation and processing environment is clean (AgBiotech Info, 1998).

It is possible to destroy the somatic (food) cell ATP by using detergents to lyse the cells and ATPase to destroy the ATP or to remove microbial cells from the sample by filtration prior to extracting their ATP. However, the most widespread application of ATP measurement is as a hygiene test to monitor the cleanliness of food production areas. For this, the surface is swabbed and the total ATP on the swab is extracted and measured. In this application, it does not matter whether the ATP is derived from food residues or from microorganisms because both are indicative of an inadequately cleaned surface (Anand, 2004).

To determine how much ATP is present in the sample being tested, scientists use the enzyme luciferase, found in firefly tails. It is this enzyme which makes fireflies glow in the dark. Luciferase reacts with ATP to form light. The amount of light produced is directly proportional to the concentration of ATP in the sample. This light-producing reaction takes only a few seconds. The light output is usually measured by a simple portable machine called luminometer. The technique has a detection limit of 1pg ATP, which is equivalent to approximately 1000 bacterial cells. It is a rapid test, taking less than one hour to complete. The light-yielding reaction is efficient, producing a single photon of light for every luciferin molecule oxidized and thus every ATP molecule used (Anand, 2004).

All living cells (eucaryotic and procaryotic) contain ATP, the high-energy intermediate that powers most energy-consuming reactions.

ATP is not found in the environment except where there are either metabolically viable cells or cells which have recently ruptured and released their ATP into the immediate bulk environment. The ATP method in general was initially developed as a chemical method for the indirect enumeration of microbial cells since all cells contain ATP.

Luciferin/Luciferase System

Luciferin is the most popular and versatile bioluminescent substrate. The firefly luciferin/luciferase bioluminescent system is found in the firefly (*Photinus pyralis*) and several other beetles. The reaction between ATP and the enzyme luciferase produces light, which is measured photometrically,

with a claimed sensitivity down to 10^{-16} moles of ATP. It is a rapid test, taking less than one hour to complete.

The firefly beetle luciferase studied initially during the 1940's by McElroy and DeLuca is used most extensively in molecular biology (Lappalainen, 2000) and biochemistry today (Gould, 1988). The introduction of firefly luciferase to molecular biology began in 1986 with the publication of a glowing tobacco plant by researchers at UC San Diego (Ow, 1986). The firefly luciferase gene was linked to a plant virus promoter, inserted into a bacterial plasmid and subsequently transfected into tobacco leaf cells in culture. Transgenic plants derived from these cultured cells stably expressed the luciferase gene. When the substrate luciferin was applied to the plants, they produced a uniform glow. For use in the laboratory, this form of light emission can yield a very sensitive nonradioactive assay. During the past fifty years, the firefly luciferase has been extensively used for sensitive applications involving measurement of ATP, including monitoring biomass, analyzing biological fluid contamination from microbes, assessing cell viability, and assaying enzymes involving ATP generation. Finally, luciferases are also successfully used as biosensors for environmental pollutants such as arsenite, mercury, lead, phenols, agrochemicals, and xenoestrogens that may act as endocrine disruptors to humans and wildlife.

The luciferin/luciferase chemical reaction has been used for years to measure the amount of ATP produced in cells and by various chemical reactions. Recently, the gene (section of DNA coding for the protein) for the luciferase enzyme has been isolated, placed in the genes of other organisms, and used to follow the synthesis and/or expression of other genes (i.e. used as a reporter gene). After cloning the firefly luciferase cDNA, scientists have used the luciferase gene as a sensitive reporter of gene expression. Some examples of these applications include using these genes to study the progression and regression of viral and bacterial disease, such as HIV and mycoplasmas, as well as the non-invasive assessment of therapeutic treatment of tumor proliferation and regression in animal models (Lundin, 1982).

Luciferase can be utilized as a reporter gene to monitor promoter response activity in bacteria, cultured cells, and transgenic plants or animals. By providing faster results, lower costs and over a 1,000-fold increase in sensitivity, the luciferase assay has largely replaced the standard 14C chloramphenicol acetyltransferase (CAT) assay.

The use of the luciferase gene as a tool for *in vivo* studies is somewhat more limited due to the inefficient entry of substrate luciferin into cells. Many approaches to solve this membrane permeability have been reported (Craig, 1991). The beetle luciferase gene *luc* has been expressed in bacteria,

yeast and plant cells and luciferase as a reporter enzyme is used in the analysis of transcription and regulatory mechanism in eukaryotic cells. Firefly luciferase has long been conjugated to antibodies and used as a label in immunoassays with luciferase as the substrate for detection.

Another important use of luciferase is in the area of hygiene monitoring. Taking advantage of the fact that all living organisms contain ATP, contamination can be detected utilizing the bioluminescent properties of the luciferin/luciferase system where ATP is required as a co-factor for luminescence. In vitro, the luciferase enzyme is assayed by addition of luciferin, ATP and magnesium ions.

The luciferin/luciferase chemical reaction

The firefly luciferase, a 62,000 dalton protein which is active as a monomer, does not require subsequent processing for its activity.

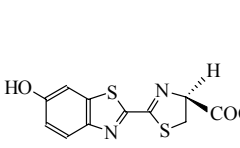
Firefly bioluminescence is a multi-step process (Equations 1-3):

$$Luc + LH_2 + ATP \xrightarrow{Mg^{2+}} Luc \cdot LH_2\text{-}AMP + PP_i \quad (1)$$

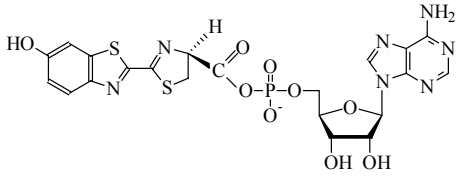
$$Luc \cdot LH_2\text{-}AMP + O_2 \longrightarrow Luc \cdot Oxyluciferin^{*} + AMP + CO_2 \quad (2)$$

$$Luc \cdot Oxyluciferin^{*} \longrightarrow Luc \cdot Oxyluciferin + light \quad (3)$$

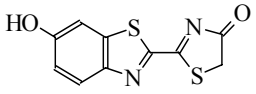
Luc is firefly luciferase; * denotes an electronic excited state.



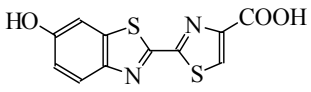
D-Firefly Luciferin (LH_2)



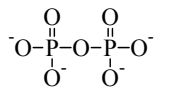
D-Luciferyl-O-AMP (LH_2-AMP)



Oxyluciferin



Dehydroluciferin (L)



Pyrophosphate (P_iP)

In the first step (Eq. 1), luciferase converts firefly D-luciferin into the corresponding enzyme-bound luciferyl adenylate. Firefly luciferase has extraordinary specificity for this nucleotide triphosphate. The adenylate is the true substrate of the subsequent oxidative chemistry. In fact, D-LH_2-AMP produced synthetically reacts with oxygen in the presence of luciferase to produce light emission identical to that obtained with the natural substrates

D-luciferin and Mg-ATP. As Equations 2 and 3 indicate, the luciferase enzyme functions as a mono-oxygenase, although it does so in a very unusual manner without the apparent involvement of a metal or cofactor. In some way that has not been yet determined, luciferase amino acid residues are recruited to promote the addition of molecular oxygen to luciferin, which is then transformed to an electronic excited state oxyluciferin molecule and carbon dioxide, each containing one oxygen atom from molecular oxygen. Visible light emission results from the rapid loss of energy of the excited state oxyluciferin molecule via a fluorescence pathway. The very high quantum yield for this process (in alkaline solution, nearly each reacted LH_2 molecule emits a photon) reflects not only efficient catalytic machinery, but also a highly favorable environment for the radiative decay of an electronic excited state.

In addition to the reactions leading to light emission, firefly luciferase also catalyzes the *in vitro* formation of the adenylate of dehydroluciferin (L-AMP) (Eq. 4), which cannot react further and potently inhibits enzyme activity. Thus, luciferase exhibits two distinct enzymatic functions: as a synthetase in the formation of an acyl adenylate, and as a mono-oxygenase.

$$\text{Luc} + \text{L} + \text{ATP} \xrightarrow{Mg^{2+}} \text{Luc}\cdot\text{L}^{-}\text{AMP} + PP_i \quad (4)$$

Also, under certain conditions LH_2-AMP, formed according to Eq. 1, may be oxidized by luciferase to produce low levels of L-AMP. Subsequently, luciferase catalyzes the transfer of the AMP moiety from L-AMP to ATP producing diadenosine tetraphosphate. These chemical transformations indicate that firefly luciferase can also function as a ligase. Furthermore, Coenzyme A can inhibit this ligase function and modulate the usual light emission kinetics, although the cofactor is not a required substrate. Coenzyme A is known to stimulate light production possibly by promoting the release of product oxyluciferin from luciferase allowing the enzyme to react again (Lee, 1970; DeLuca, 1974, 1976; Lemasters, 1977; Branchini, 2002).

Firefly luciferase has the highest efficiency of any known bioluminescence reaction with a quantum yield of 0.88. The wavelength of light given off is between 510 and 670 nanometers (pale yellow to reddish green color). The intensity and duration of the light emitted are a function of reaction conditions, reagent purity and other factors. Instruments ranging from a simple spectrophotometer to a bioluminometer or special video cameras may be employed for light measurement. In general, the intensity of light emission may be conveniently recorded after a 20-second time period. If automated sample injection capacity is available, a three-second-time period may be appropriate.

At the optimum reaction pH of 7.8, light emission peaks at 562 nm. With pH shifts to more acidic ranges, a second emission peak at 616 nm increases until at pH 5.4 it reaches a maximum and there is no measurable 562 nm emission. This environment pH dependent change in spectral emission is due to protonation of the oxyluciferin reactive species. Light emission peaks in about one second, and then decays rapidly with a typical half-life of one minute. Arsenate will extend the half-life substantially but with diminished sensitivity for the detection of ATP.

As with many enzymes, firefly luciferase follows Michaelis-Menten kinetics and maximum light output is not achieved until excess substrate (above the Km) luciferin and co-factor concentrations exist. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. The Km values for Photinus pyralis luciferase with D-luciferin and ATP at pH 7.8 are 1.7×10^{-5} and 3.4×10^{-4} respectively (Lemasters, 1977). These concentrations should be considered when designing a luciferase assay; it is also to be noted that these values are dependent on pH.

Optimum temperature for the luciferase/luciferin reaction is 28°C. The reaction slows perceptibly at higher temperatures. Vigorous agitation is to be avoided, as it may denature the luciferase enzyme.

Applications of ATP - bioluminescence in food industry

There are essentially three areas of application: hygiene monitoring, testing liquids such as final rinses from Clean in Place systems and assessing the bacteriological quality of foods. The first two applications measure the total ATP content of a sample.

This will include eucaryotic and microbial ATP. In contrast, for assessing the bacteriological quality of foods only the microbial ATP must be measured and this requires selective extraction procedure. First, non-microbial ATP is extracted with a non-ionic detergent (e.g. Triton X-100) and then destroyed by treating with a high level of ATPase for 5 minutes. The microbial ATP is extracted using trichloracetic acid (5%), an organic solvent (ethanol, acetone, chloroform), or a cationic detergent.

The ATP method has been used to evaluate microbial loads in e.g. meat, milk, water, and fruit juice samples in a winery and a brewery, sweeteners and syrups (Fung, 1997). Much interest has been developed also in using ATP estimation not for total viable cell counts but as a sanitation check including also the verification of somatic cells presence on a surface. This version of the ATP bioluminescence method based on detecting all ATP on a surface provides an indication of cleanliness detecting also ATP of somatic

origin (that the traditional plate count method does not detect), instead of only of microbial origin (Chen, 2000; Quinn, 2002; Paez, 2003). Samples for assessing surface hygiene by this method can be obtained by swabbing the surface, or by taking aliquots of the rinse water.

ATP bioluminescence technology can be applied to bring a rapid microbial test to the dairy industry, allowing more effective management of the freshness/quality balance. The ATP pasteurized milk screen is a tool for dairies to quickly identify a problem when significant levels of microorganisms have somehow found their way into finished product. Armed with this valuable information, dairy processors can begin the response process quickly, potentially sparing additional production days exposed to the source of contamination. When corrective action can start days sooner, shelf life quality will certainly increase.

ATP bioluminescence has the potential to be a useful tool to evaluate the effectiveness of cleaning procedures used on the milking machines. The ATP method appeared to be a more sensitive method to detect differences in cleaning effectiveness than bulk tank culture method (Reinemann, 1997; Murphy, 1998; Kyriakides, 1992; Bell, 1994).

Water quality control, for any of the uses in a food plant, is necessary to avoid possible risks to consumer's health. In addition, it can reduce negative effects on facilities and processes such as corrosion, deposit and sediment formation and attend the quality criteria of each finished product in the food industry. Water may be used as a component of the final products and as part of the manufacturing process, e.g., transport and cooling. In addition, it is in direct or indirect contact with foodstuff as well as used to wash hands, equipment, utensils and facilities.

To assess the microbiological quality of water, the conventional techniques (plate count agar and coliform tests) are laborious and time consuming. To attend the food industries demands, rapid and sensitive methods, like ATP-bioluminescence technique, have been developed (Costa, 2004). This technique was also used to evaluate the water quality of a public water treatment system (Davenport, 2002). Most systems operate with a certain baseline value for total ATP. Once this baseline number is determined, a fast change in the ATP reading can show either the instantaneous effect of a treatment chemical or the long-term effect of a treatment chemical on the overall fouling potential of a system.

The light output (directly proportional to the concentration of ATP in the sample) measured by the most used luminometers is expressed as relative light units (RLU).

Some typical RLU values are (Relenyi, 2000): Drinking fountain in an industrial plant 200 RLU; Geothermal formation water used for reinjection

1000 RLU; Aquifer source water for refinery 300 RLU; Clean recirculating system 3000 RLU; Bottled water 212 RLU; Household drinking water 5 RLU; Sterile water for babies 1 RLU; Odorous well water 310,000 RLU.

These values are shown to demonstrate the fact that an ATP measurement in conjunction with other system parameters can be used as a baseline measurement of acceptable performance. For comparison, critical food preparation areas in the food processing areas are considered clean when RLU levels are <150 RLU (Costa, 2004).

Based on these results and on experience gained in other industries, pass/caution/fail limits were set as follows:

RLU Reading	Hygiene Rating
≤100	Pass
101 – 499	Caution
≥500	Fail

The ATP bioluminescence testing system is very simple to use and highly sensitive (usually being able to detect less than 3 x 10^{-14} moles of ATP, which is around the usual ATP content of a few microlitres of rough beer). All staff could perform the test and it's no need for laboratory facilities. The ATP test has also been shown to be fairly insensitive to chemical interference from detergents and disinfectants, which have sometimes caused problems with other testing systems (Ehrenfeld, 1996; Lysert, 1976).

Conclusions

ATP bioluminescence is one of the quickest and most useful methods of finding and tracking the source of bacteria that can contaminate food like beef, pork, milk and poultry and to assess the microbiological quality of water. Because ATP bioluminescence can indicate the presence of food residues, it is also particularly useful for rapidly determining if the food preparation and processing environment is clean. The advantages of the ATP method are: quick, highly sensitive, simple to use, cheap to use. It is also an evaluation tool to validate biocides and related chemicals.

References

AgBiotech Infosource (1998). Biotechnology in Food Safety: Protecting Consumers. http://www.agwest.sk.ca/publications/infosource/inf_mar98.php

Anand, S.K., Griffiths, M. W. (2004). Inside Microbiology: Advances in Biotechniques Used in Quality Assurance of Food Products. www.foodsafetymagazine.com/issues/0312/colmicro0312.htm

Bell, C., Stallard, P.A., Brown, S.E., Standley, J.T.E. (1994). ATP-bioluminescence techniques for assessing the hygiene condition of milk transport tankers. *Int. Dairy Journal*. 4, 629-640.

Branchini, B. (2002). Firefly bioluminescence. http://modares.ac.ir/sci/saman_h/Pages/Firefly.htm.

Chen, J. (2000). ATP bioluminescence: A rapid indicator for environmental hygiene and microbial quality of meats. *Dairy, Food Environ. Sanit.,* 20, 617-620.

Costa, P.D., de Andrade, N.J., Passos, F.J.V., Brandão, S.C.C., Rodrigues, C.G.F. (2004). ATP-bioluminescence as a technique to evaluate the microbiological quality of water in food industry. *Braz. Arch. Biol. Technol.,* 47 (3), 399-405.

Craig, F.F., Simmonds, A.C., Watmore, D., McCapra, F. (1991). Membrane-permeable luciferin esters for assay of firefly luciferase in live intact cells. *Biochem. J.,* 276, 637-641.

Davenport, K., Lang, G., Phillips, R. (2002). Applications of ATP Bioluminescence in the monitoring of recreational pool water. www.biotrace.co.uk/uploads/documents.

DeLuca, M., McElroy, W.D. (1974). Kinetics of the firefly luciferase catalyzed reactions. *Biochemistry,* 13 (5), 921-925.

DeLuca, M. (1976) Firefly luciferase. *Adv. Enzymol.* 44, 37-68.

Ehrenfeld, E.E., Scheld, J., Miller, S.A., Carpenter, C.R. (1996). Use of ATP-bioluminescence in the brewing industry. *Tech. Q. Master Brew. Assoc. Am.,* 33 (1), 59-62.

Fung, D. Y. C. (1997). Overview of rapid methods of microbiological analysis. In: Tortorello, M. L., Gendel, S. M. (eds.) *Food Microbiological Analysis*, (pp. 1-25). New York: Marcel Dekker, Inc.

Gould, S.J., Subramani, S. (1988). Firefly luciferase as a tool in molecular and cell biology. *Analyt. Biochem*., 175, 5-13.

Gracias, K.S., McKillip, J.L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, 50, 883-890.

Griffiths, M. W. (1993). Applications of bioluminescence in the dairy industry. *J Dairy Sci.*, 76, 3118-3125.

Griffiths, M.W. (1996). The role of ATP Bioluminescence in the food industry: New light on old problems. *Food Technology*, 50, 62-73.

Kyriakides, A. (1992). ATP bioluminescence applications for microbiological quality control in the dairy industry. *Journal of the Society of Dairy Technology*, 45 (4), 91-93.

Lappalainen, J., Loikkanen, S., Havana, M., Karp, M., Sjöberg, A-M., Wirtanen, G. (2000). Microbial testing methods for detection of residual cleaning agents and disinfectants – Prevention of ATP bioluminescence measurement errors in the food industry. *J. Food Prot.*, 63, 210-215.

Lee, R.T., Denburg, J.L., McElroy, W.D. (1970). Substrate-binding properties of firefly luciferase. *Arch. Biochem. Biophys*., 141, 38-52.

Lemasters, J.J., Hackenbrock, C.R. (1977). Kinetics of product inhibition during firefly luciferase luminescence. *Biochemistry*, 16 (3), 445-447.

Lundin, A. (1982). Applications of firefly luciferase. In M. Serio, M. Pazzagli, *Luminescent Assays: Perspectives in Endocrinology and Clinical* Chemistry, (pp: 29-45). New York: Raven Press.

Lysert, D.W., Kovecses, F., Morrison, N.M. (1976). A firefly bioluminescence ATP assay method for rapid detection and enumeration of brewery microorganisms, *ASBC J*., 34, 145, 1976.

Murphy, S.C., Kozlowski, S. M., Bandler, D. K., Boor, K. J. (1998). Evaluation of ATP-bioluminescence hygiene monitoring for trouble-shooting fluid milk shelf-life problems. *J. Dairy Sci.*, 81, 817-820.

Nilsson, L., Molin, O., Aansehn, S. (1984). Rapid detection of bacteriemia by bioluminescent assay of bacterial ATP. In L.J. Kricka, *Anal Appl Biolumin Chemilumin* (Proc Int Symp) 3rd, (pp 25-28). London: Academic Press.

Orth, R., Steigert, M. (1996). Hygiene monitoring: practical experience in the ATP-bioluminescence measuring method to control hygiene after cleaning of a meat processing plant. *Fleischwirtschaft*, 76, 1143-1144.

Ow, D.W., Wood, K.V., DeLuca, M., de Wet, J.R., Helinski, D.R., Howell, S.H. (1986). Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science*, 234, 856-859.

Páez, R., Taverna, M., Cuarlón, V., Cuatrin, A., Etcheverry, F., Da Costa, L. H. (2003). Application of ATP-bioluminescence technique for assessing cleanliness of milking equipment, bulk tank and milk transport tanker. *Food Protection Trends,* 23, 308 – 314.

Poulis, J. A., de Pijper, M., Mossel, D. A., Dekkers, P. A. (1993). Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminescence technique combined with the tissue fluid contamination test and a conventional microbiological method. *Int. J. Food Microbiol.*, 20, 109-116.

Quinn, B. P., Mariott, N. G., Alvarado, C. Z., Eigel, W. N., Wang, H. (2002) HACCP plan assessment of Virginia meat and poultry processing plants. *Dairy, Food Environ. Sanit.*, 22, 858-867.

Reinemann, D.J., Mein, G.A., Bray, D.R., Reid, D., Britt, J.S. (1997). Troubleshooting High Bacteria counts in Farm Milk. *Proc. 36th annual meeting of the National Mastitis council, 16-19 Feb. 1997*.

Relenyi, A.G. (2000). Organic Film and General Organic Fouling Indices: ATP, Dip Slides, and Agra Film. www.awt.org/members/publications/analyst/2000/ Spring/F3-organic%20Film.htm.