African Journal of Microbiology Research Vol. 5(24), pp. 4218-4225, 30 October, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.927
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Improvement of bioluminescent assay by combining with the cell growth kinetics for coliforms detection

Anna Ilyina[1]*, Anita J. Casas-Reyes[1], Karla M. Gregorio-Jauregui[1], Erika Nava-Reyna[1], Jose L. Martinez-Hernandez[1], Nagamani Balagurusamy[2], Federico Cerda-Ramirez[3] and Yolanda Garza-Garcia[1]

[1]Chemistry School, Autonomous University of Coahuila, Saltillo, Coahuila, México.
[2]School of Biological Sciences, Autonomous University of Coahuila, Saltillo, Coahuila, México.
[3]Applied Chemistry Investigation Center, Saltillo, Coahuila, Mexico.

Accepted 30 September, 2011

The present study focuses on the improvement of limitations of the bioluminescent ATP assay. An attempt was made to improve on the following: non-selective detection, limited cell population density quantification, and to distinguish between bioluminescent noises data and cell ATP, as a suitable technique for coliforms and *Escherichia coli* detection, as well as other cell strains. Results were achieved by combining growth kinetics of the cell population exponential phase, in 2% brilliant green lactose bile broth with bioluminescent assay, based on the reaction catalyzed by firefly luciferase. The equations applied to the description of kinetics on exponential growth phase were able to estimate the relative light units (RLU_0) corresponding to initial adenosine triphosphate (ATP_0) concentration. The developed equations also help to distinguish between background RLU and RLU_0 corresponding to cell ATP, and to analyze the kinetics with and without lag period in order to estimate RLU_0. The results demonstrated a good correlation with data obtained by standard methods for coliforms detection. The quantification of *E. coli* and coliforms could be performed within a period of 6 h at 3.24×10^5 and 1.12×10^3 CFU/mL from the calculation of initial ATP concentrations at 19 and 0.067 pmol/mL, respectively. Monitoring of growth kinetic parameters along with the bioluminescent detection of microorganisms in the selective medium during shorter test time has potential for their selective and sensitive quantification. The mathematical approach may be extended to the analysis of experimental data obtained by other techniques based on the cell metabolite detection.

Key words: Bioluminescence, coliforms, *Escherichia coli*, exponential phase of growth, adenosine triphosphate (ATP) detection.

INTRODUCTION

Adenosine triphosphate (ATP) measurement using bio-luminescent assay is widely employed for the evaluation of bacteriological quality of food and water, as ATP is only found in living cells. The main reasons for the use of extraction and analysis methods are that they are rapid, specific and can be easily reproduced. However, this method is not applied for selective detection and/or quantification of specific bacteria. The problem, that limits the application of the bioluminescence assay for such detection, is the absence of selectivity in the ATP release process, which is unable to distinguish the source of ATP. However, the necessity for rapid detection methods of coliforms, *Escherichia coli* (as bio-indicator of fecal coliforms) and other bio-indicator organisms is gaining wide importance. Several specific rapid methods have been reported (Houng et al., 1997; Blasco et al., 1998; Lee and Deininger, 2004; Regnault et al., 2000; Ivanova et al., 2006). Since these methods are highly specific, they require advanced laboratory facilities and trained staff to carry out these techniques.

The firefly luciferase-based (bioluminescence) assay for detection of ATP is a well-established method and

*Corresponding author. E-mail: anna_ilina@hotmail.com. Tel: +52-844-415-95-34.

has been used as a rapid way to monitor the hygiene of food and nonfood contact surfaces (Satoh et al., 2004). This assay technique, however, has a detection limit of approximately 10^5 colony forming units (CFUs) of *E. coli* per assay, which is not sensitive enough for some applications. ATP is the high-energy molecule that plays an important role in metabolism; therefore the development of specific methods, to detect very low concentrations of ATP, is very important in many areas of pure and applied biochemistry.

The bioluminescence detection has been improved by using more sophisticated and more expensive instruments in order to increase the sensitivity and rapidity of detection (Masuko et al., 1992). The background noise due to the presence of extracellular ATP and instrument noise in assaying procedures has been a major impediment in achieving higher sensitivity for detection of intracellular ATP (Satoh et al., 2004). Sakakibara et al. (2003) had improved the filter-based bioluminescence assay with the concomitant use of enzymes to reduce the extracellular ATP background noise, enzymatic ATP cycling and the bioluminescence reaction, and also was able to detect a single bacterial cell. Satoh et al. (2004) developed an effective method of treating biological samples by employing a combination of adenylate kinase, pyruvate kinase and polyphosphate kinase (Satoh et al., 2004).

They were able to detect very low levels of ATP without using a photon detector. This assay, is approximately 10,000-fold more sensitive to ATP than the bioluminescence assay without ATP ampli-fication, and allowed to detect bacterial contamination as low as one CFU of *E. coli* per assay. However, this technique was not selective for definite types of bacteria.

Although concomitant used ATP cycling and the bioluminescence assay (Sakakibara et al., 1999) increased luminescence by signal integration without forfeiting the linear correlation between luminescence and ATP concentration, the background noise was also amplified. Hence it was difficult to enumerate cells precisely by the ATP amplification method.

Apart from the difficulties of improving the sensitivity of detection, Selan et al. (1992) reported incidence of false-negative tests in the conventional standard methods employing luciferin-luciferase-ATP-dependent reaction for measurement of ATP. One of the causes of such false-negative results is the different ATP concentration in distinct type of bacterial species and non-selectivity of assay. Some strains have a minor ATP content or interference of some compounds with the luciferin-luciferase reaction (Masuko et al., 1992), which led to these altered results. Clear differences in bioluminescence readings of bacterial cultures in different phases of growth were also reported (Masuko et al., 1992; Selan et al., 1992). Since low relative light unit (RLU) values are frequently comparable to the mean of background noise value, it is difficult to distinguish between one and another. Some authors (Selan et al., 1992; Varfolomeyev and Gurevich, 1999) suggest the need to develop a procedure to distinguish background noise (RLU_x) from bacteria-free samples and contaminated specimens, instead of using a single breakpoint value, as suggested by other authors (Masuko et al., 1992).

This paper aimed to present the results of the work carried out in order to improve the sensitivity, selectivity and accuracy of bioluminescent assay without using costly instruments or enzyme coupling reactions, used for coliforms and *E. coli* detection. The goals of the present study are: 1) to analyze the coliforms and *E. coli* growth kinetics by means of ATP detection by the bioluminescent assay in the 2% brilliant green lactose bile broth as a medium selective for coliforms; 2) to propose a mathematical method useful to distinguish between background noise and ATP-related values as well as for quantification of low coliforms population density in the water samples; 3) to compare results obtained using developed approach with the conventional standard methods for the coliforms detection.

MATERIALS AND METHODS

Growth kinetics of *E. coli*

In this study, *E. coli* (BD Bactrol plus ATCC 25922 Bioscience, USA) strain was used and preserved according to the instructions provided by the supplier. The strain was maintained at 2% brilliant green brilliant bile agar (Bioxon, Mexico). The inoculum was prepared by cell incubation on 2% brilliant green brilliant bile broth (Sigma, USA) at 37°C for 8 h. The water sample from the water well was obtained near Saltillo City (Coahuila, Mexico) by following the official regulations (USEPA, 1983).

The strain or water sample (0.1 mL) was inoculated in tubes (10 mL) containing 7 mL of culture broth mentioned above, and incubated at 37°C for 8 h to study the kinetics of growth. The growth of *E. coli* was determined periodically by means of bioluminescent assay.

The population of *E. coli* was determined by Most Probable Number (MPN) technique, using 2% brilliant green brilliant bile broth, and CFU/mL, using violet red bile agar (VRBL), by following the standard methods (APHA, 1998). All assays were performed in triplicate under similar conditions.

Bioluminescent assay for monitoring the *E. coli* growth kinetics

E. coli and coliforms were grown and multiplied under conditions described above. In order to avoid the interferences of the components of the culture broth in bioluminescence detection, the cells were separated from the culture broth by means of centrifugation at 4,000 g for 10 min at 4°C. The cell pellets were broken with 90 µl DMSO (Fisher Biotech, F-Scientific USA) and the released ATP were detected by bioluminescent method. The bioluminescent ATP detection was based on the reaction catalyzed by firefly luciferase (EC 1.13.12.7), and the assays were performed according to the techniques outlined in the luciferin-luciferase kit (Sigma, USA). The light emission was detected in a TD-20/20 luminometer (Promega, Madison, USA) with integration over 10 s and initially was measured as a Relative Light Units (RLU). The

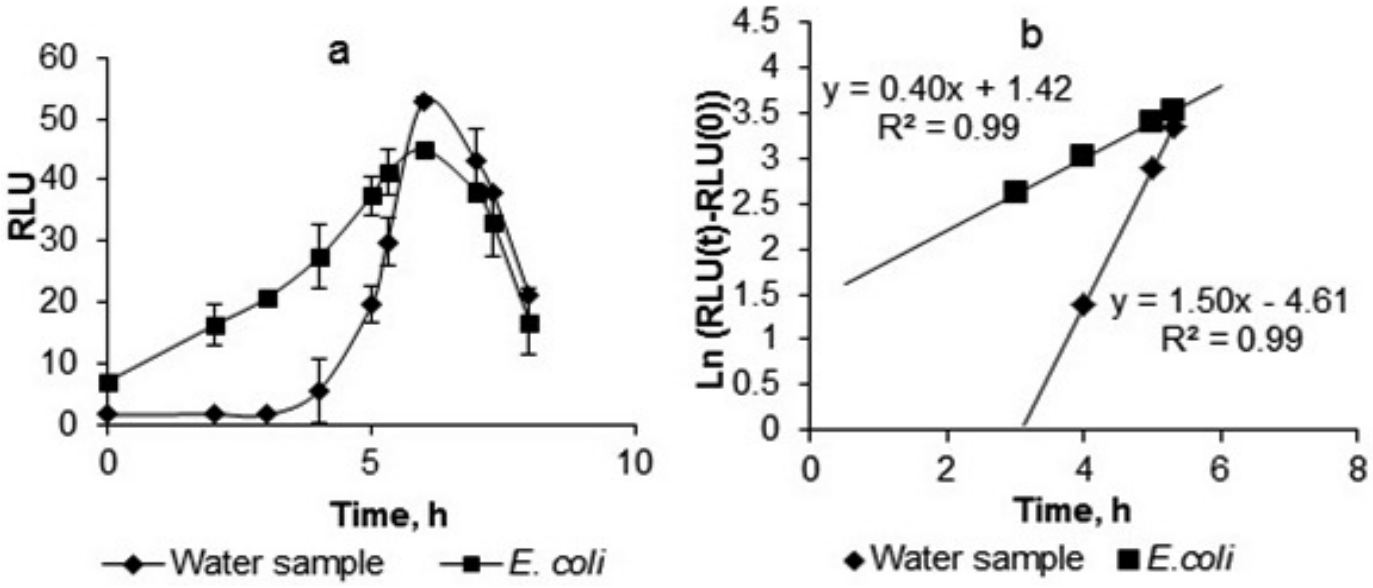

Figure 1. Growth kinetics of *E. coli* ATCC 25952 (-■-) and coliforms from the water sample (-♦-) in 2% brilliant green bile broth: a, - Relative Light Units (RLU) detected by bioluminescent assay; b, - Linearization in semilogarithmic coordinates.

activity of the luciferin-luciferase was quantified against a standard curve obtained by using different ATP (Sigma, USA) concentrations. Based on Malthusian growth model (Varfolomeyev and Gurevich, 1999), obtained values were plotted on semi-logarithmic coordinates to analyze kinetic parameters. The growth kinetics data were analyzed by means of multifactor variance analysis and the statistical significance was determined at 95% confidence limits.

Assay to establish the relationship between ATP, CFU and MPN

To define the relation between ATP and CFU, as well as with MPN, *E. coli* suspensions at different dilutions were used. Techniques described above were performed to quantify ATP and cell population density. The ATP/CFU and ATP/MPN values were calculated from the slopes obtained in ATP (mol/mL) *vs* CFU/mL and ATP (mol/mL) *vs* MPN/100 mL coordinates, respectively. All assays were carried out in triplicate. Standard deviation and mean values were calculated for both bacteria counts, and bioluminescent ATP determination.

RESULTS

The present investigation revealed that the cells proliferation on selective media allowed significant increase in their number, as well as discriminating detection of cells that could grow in this media. Thus from few cells not detectable by bioluminescent technique, a quantifiable population might be obtained. The kinetic study verified that the detected values were associated with viable cells, which could grow, and solved the problem to distinguish between bioluminescent noises data and cell ATP.

The typical kinetic curves obtained by bioluminescent assay with the water sample and standard *E. coli* strain are presented in Figure 1a. The presence of the exponential phase was confirmed by linearization of initial growth data in semi-logarithmic coordinates (Figure 1b).

The exponential growth model was described by the equation $RLU(t_i) = RLU_0 \exp(\mu t_i)$, where $RLU(t_i)$ – relative light units detected at an arbitrary time, the value RLU_0 corresponding to RLU at zero time (the initial bacteria population) and μ was organism's specific growth rate. Application of semi-logarithmic coordinates led to μ value estimation as a slope of the linear function and Ln (RLU_0) value as *y*-intercept (Figure 1b). However, the simple exponential equation might be complicated by the presence of background (RLU_x) related to equipment noise or unspecific ATP level (Figure 1), as well as by induction period (τ):

$$RLU(t_i) = RLU_0 e^{\mu(t_e + \tau)} + RLU_x,$$

Where: t_e corresponding to exponential growth time and τ to lag phase, therefore $t_i = t_e + \tau$.

In the present study some equations were derived from Malthusian growth model to define the mathematical method for quantification of initial cell population from the exponential growth phase (Table 1) based on the experimental results obtained using bioluminescent assay. The specific growth rates (μ) applied for calculations were 0.4 and 1.5 h^{-1} for *E. coli* y coliforms from the water sample, respectively (Figure 1b).

The mathematical procedure (Varfolomeyev and Gurevich, 1999) for induction period estimation considered that for the exponential phase growth the equation:

$$Ln((RLU(t_i) - RLU_x)/ RLU_0) = \mu(t_i - \tau)$$

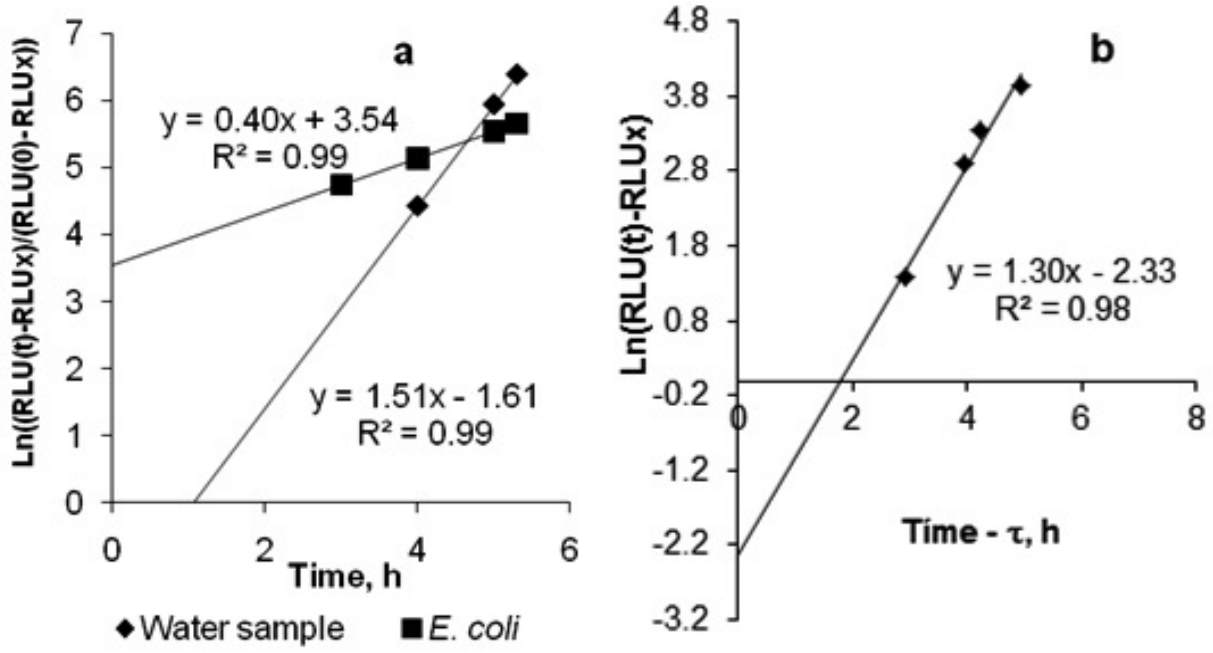

Figure 2. Linearization of experimental data **(a)** - en semi-logarithmic coordinates considering RLUx to define the presence of lag period; **(b)-** data for water sample after considering lag period (τ) to define the RLU_0).

might be applied. Positive *x*-intercept value indicated to presence of the lag phase. Thus, when the value described by Ln ((RLU (t_i) - RLU_x)/ RLU_0) = 0 (*x*-intercept condition), τ was equal to time value in this point (Figure 2a). This behavior was observed for assay performed with the water sample but not for *E. coli* assay (Figure 2a).

The results obtained by application of the equations to experimental data are presented in Figures 1 and 2, as well as in the Table 2. The linearization of experimental data in semi-logarithmic coordinates considering the RLU_x value quantified by equation described in the Table 1A and the lag phase period τ (Figure 2b) aided in the determination of RLU_0 corresponding to ATP_0 (Table 2). The RLU_x value quantified for *E. coli* was close to RLU (0) and was detected in the first measurement without incubation (Table 2), at the same time the linear function was similar to presented in the Figure 1b, which was applied in RLU_0 calculation.

Figure 3 presents calibration plots applied to diverse conversions. To define the conversion coefficients, the relations between ATP mol/mL and CFU/mL as well as MPN/100 mL were estimated by means of independent experiments (Figures 3a and 3b, respectively) as described above. The calibration curve useful to conversion of RLU to ATP value (Figure 3c) demonstrated that the minimum detected ATP concentration is 10^{-10} M.

The results calculated by kinetic approach demonstrated a good correlation with the results obtained by means of a standard microbiological technique (Table 2), while the values quantified from direct measurement (Table 2) were significantly higher: more than 2 times and 51 times for assay with *E. coli* and water sample, respectively.

DISCUSSION

It was observed that the ATP concentration decreased during the stationary phase (Figure 1a). This is in agreement with the results reported earlier by Nuzback et al. (1983). They recorded an immediate decline in ATP during stationary phase and further reported that there is no correlation between the population count of the bacteria and the ATP concentration during the stationary phase. Murray et al. (2003) demonstrated that the decline in ATP levels and the reduction in rRNA transcription (*rrnB* P1 promoter activity) during the stationary phase are related (Murray et al., 2003). This clearly indicated that ATP is not a static parameter and is related to the metabolic status of the viable cells.

The method for determination of the lag period has been described previously (Varfolomeyev and Gurevich, 1999). Lag phase was estimated as the abscissa in the *x*-intersect point of linear function in semi-logarithmic coordinates presented in the Figure 2a. The positive abscissa value indicated the presence of the lag phase that was observed only in the case of assay performed with the water sample. The line obtained from *E. coli* assay data was characterized by negative *x*-intersect point that indicated the absence of the lag phase

Table 1. Mathematical description of equations derived to calculate initial RLU_0 related to the initial ATP_0 concentration.

A. Equation for RLU_x (noise RLU) estimation	For the exponential growth phase: $RLU(t_i)=RLU_0 e^{\mu(t-\tau)} + RLU_x$ where t_i is a incubation time and τ is a lag phase time, μ value is estimated from linear function in semi-logarithmic coordinates. RLU_0 is considered as a value corresponding to initial cell ATP_0. *So, RLU_x may be defined from equitation's system:* $RLU(t_1) = RLU_0 e^{\mu(t_1-\tau)} + RLU_x$ $RLU(t_2) = RLU_0 e^{\mu(t_2-\tau)} + RLU_x$ *where experimental data corresponding to exponential phase of growth at different incubation times (t_1 and t_2) are used. From the second equation is obtained:* $RLU_0=(RLU(t_2) - RLU_x)/e^{\mu(t_2-\tau)}$ *Introduction of this function in first equation leads to:* $RLU(t_1)= RLU(t_2) e^{\mu(t_1-t_2)} - RLUx (1-e^{\mu(t_1-t_2)})$ *where RLUx is the single unknown. So, the RLU_x may be estimated as* $RLU_x = (RLU(t_1) - RLU(t_2)e^{\mu(t_1-t_2)})/(1 - e^{\mu(t_1-t_2)})$
B. Equation for RLU_0 estimation in the absence of lag period (τ)	*For exponential phase of growth:* $RLU(t_i) = RLU_0 e^{\mu(t_i)} + RLU_x$ *where RLU_x represented the background RLU (free of RLU corresponded to initial concentration of bacteria) and $RLU(t_i)$ is detected in time t_i as experimental value.* *So,* $RLU(t_i) - RLU_x = RLU_0 e^{\mu(t_i)}$ $Ln(RLU(t_i)- RLU_x) = Ln(RLU_0) + \mu t_i$ *is linear function in semi-logarithmic coordinates and* $RLU_0 = e^{\text{(ordinate of intersection with OY axis)}}$
C. Equation for RLU_0 estimation in the presence of lag period (τ)	$RLU(t_i)=RLU_0 e^{\mu(t_i-\tau)} + RLU_x$ *where τ is a lag period time, which is approximately estimated as the abscissa of point of intersection of linear function with OX axis in coordinates Ln ((RLU (t_i)-RLU_x)/(RLU(0) - RLU_x)) vs time, where RLU(0) is the first bioluminescence measurement before incubation and RLU(0)-RLU_x is estimation of RLU_0.* $RLU(t_i) - RLU_x = RLU_0 e^{\mu(t_i-\tau)}$; $Ln(RLU(t_i)- RLU_x) = Ln(RLU_0) + \mu(t_i - \tau)$ *is linear function in semi-logarithmic coordinates where OX axis is ($t-\tau$).* So, $RLU_0 = e^{\text{(ordinate of intersection with OY axis)}}$

(Varfolomeyev and Gurevich, 1999).

The derived equations (Table 1) were able to estimate the RLU_0 corresponding to initial ATP_0 concentration, which in the present study are undetectable directly due to the low initial cell population density and the background noise presence. The developed equation also helped to distinguish between background RLU (RLU_x) and RLU_0 corresponding to cell ATP, and to analyze the kinetics with and without the lag period (τ) in order to estimate RLU_0 (Table 1).

The analysis of kinetic data of *E. coli* growth without the lag period (Figure 1b) is simpler than the data with the lag period (Figure 2b). The calculated RLU_x was positive and was similar to the RLU (0) detected at zero time. Although the detected signal is low, it is close to ATP detection limit (10^{-10} M). Calculated RLU_x was used to estimate RLU_0 (Figure 1b and Table 2) applying the equations presented in Table 1B.

Table 2. Calculated data of the initial coliforms population estimation in samples using standard *E. coli* strain and water sample.

Essay		RLU(0) Mean+/- Standard deviation	RLU_x	τ, h	RLU_0	ATP_0 pmol/mL	Values calculated from ATP-assay		Microbiological assay data	
							CFU/mL	MPN/ 100 mL	CFU/mL	MPN/ 100 mL
E. coli	Direct assay	7.12+/-0.12	0	0	7.12	44	7.35×10^5	7.9×10^7	3.05×10^5	3.00×10^7
	Kinetic assay		7.0+/-0.01	0	$e^{1.42} = 4.1$	19	3.24×10^5	3.48×10^7		
Water sample	Direct assay	1.59+/-0.12	0	0	1.59	4.6	7.67×10^4	8.25×10^6	1.50×10^3	2.00×10^5
	Kinetic assay		1.5+/-0.06	1.1	$e^{-2.33} = 0.97$	0.067	1.12×10^3	1.21×10^5		

To calculate the ATP_0 (M) the dilution grade (=10) with DMSO and equation of calibration plot $Log_{10}RLU = 0.67\ Log_{10}\ ATP + 7.75$ were applied. The conversion coefficients from ATP_0 mol/mL to CFU/mL and MPN/100 mL were 5.96E-19 mol/CFU and 5.54E-21 mol x 100/MPN, respectively. These values were defined by means of independent experiments (Figure 4).

The lag period (τ) is related to the adaptation of the bacterial cell to its changed growth condition and without any significant growth. The level of RLU detected in the water sample was very low and it was difficult to associate it with a real ATP without kinetic analysis. The RLU (0) value is correlated with the quantified RLU_x (Table 2), indicating that the initial ATP concentration is lower than limit of ATP detection (10^{-10} M).

The signal RLU (0) detected in bioluminescent assay before the initial time of cell incubation was measured after luminometer calibration to zero by means of the assay without cells. In the standard bioluminescent assay it may be interpreted as signal corresponding to the initial ATP of the cells. The applied mathematical analysis of obtained kinetic data demonstrated that the detected signal is a background noise of the luminometer, or the ATP levels of dead cells, or cells different to coliforms presented in applied cell suspension. It may be confirmed by comparison of the cell count quantified by RLU conversion and standard microbiological tests (Table 2). The values quantified from the direct measurement of bioluminescence were significantly higher than the number of presented microorganisms.

These results explained previously reported observations (Murphy et al., 1998; Odebrecht et al., 2000; Costa et al., 2006). For example, ATP-bioluminescence assay was used to evaluate the cleaning and sanitizing procedures of stainless steel milk contact surfaces. The results demonstrated that there is no agreement among the techniques for classification of hygienic conditions of the evaluated surfaces, showing a non-direct relation between RLU and CFU/cm^2. The ATP-bioluminescence method showed that 100% of the surfaces were under an inadequate hygiene conditions, while the plate count method detected only 50%, based on the APHA's recommendation, and 33%, based on the WHO's recommendation (Costa et al., 2006). Odebretch et al. (2000) evaluated the hygiene conditions of equipment surface in breweries applying bioluminescence methods and found that RLU measurement did not correlate with the microbial counts.

In the method of combining ATP bioluminescence with growth kinetics and by using the derived equations, it was possible to detect low cell population densities. Starting from any cell number, for the studied case, the water sample range was 10^3 cells per mL, it was possible to increase cell quantity from undetectable to detectable by bioluminescent technique due to cell multiplication. It is evident that the detection limit of cell count may be decreased to a lower level, but the test time needs to be increased in this case. For example, to obtain 10^5 cells from one cell, the waiting time is 29 h for *E. coli* and 8 h for coliform water sample, taken in account the specific growth rate determined in tests as 0.4 and 1.5 h^{-1}, respectively. The major advantages of this technique based on bioluminescence are the versatility, non-invasiveness, reproducibility, high rate and ease of assay performance with high sensitivity and lower cost (Weinrich et al., 2009).

This method is favorable to the method of detections using immunoassays, which typically detected in the range from10^4 to 10^6 of cells per mL (Choi et al., 1992; Labadie and Desnier, 1992) and was not able to differentiate between viable cells and antigens of dead cells. Moreover the assay period can be reduced in comparison with microbiological techniques, and selectivity can be increased by the use of the selective media in comparison with other bioluminescent techniques. Standard methods of cultivation need a longer time to detect the presence of coliforms, whereas

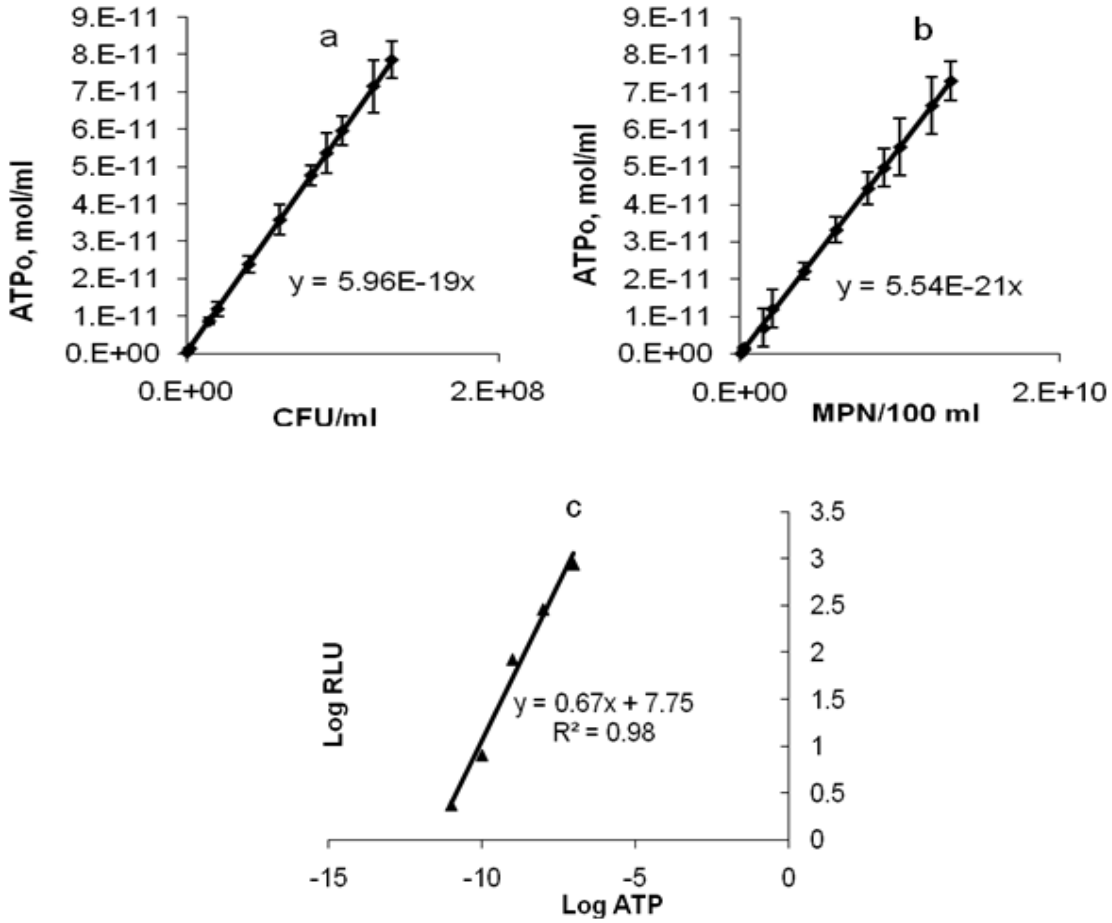

Figure 3. Calibration plots applied to define the relations between: ATP and CFU (a), ATP and MPN (b), as well as RLU and ATP (c).

in this method, the results can be obtained within a period of 6 h before the onset of stationary phase of growth in selective medium.

Conclusions

The potential of applying the growth kinetic parameters along with bioluminescent detection of *E. coli* and coliforms in the selective media for their rapid and sensitive quantification is revealed in the present investigation. The mathematical approach may be extended to the analysis of experimental data obtained by other techniques based on cell metabolite detection.

ACKNOWLEDGEMENTS

The authors are grateful for the Grant SEMARNAT-CONACYT 2002-C01-0152-A1 and the valuable help of M.S. Schluraff A. and Ph.D. Troitsky Y. for reviewing this paper for publication.

REFERENCES

APHA (1998). Standard Methods for the Examination of Water and Wastewater, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Blasco R, Murphy MJ, Sanders MF, Squirrell DJ (1998). Specific assays for bacteria using phage mediated release of adenylate kinase. J. Appl. Microbiol., 84: 661-666.

Choi D, Tang RSW, Ng NH (1992). Sandwich capture ELISA by a murine monoclonal antibody against a genus-specific LPS epitope for the detection of different common serotypes of salmonellas. J. Appl. Bacteriol., 72: 134-138.

Costa PD, Andrade NJ, Cardoso-Brandão SC, Vieira-Passos FJ, Ferreira-Soares NF (2006). ATP-bioluminescence assay as an alternative for hygiene-monitoringprocedures of stainless steel milk contact surfaces. Braz. J. Microbiol., 37: 345-349.

Houng HSH, Sethabutr O, Echeverria P (1997). A simple polymerase chain reaction technique to detect and differentiate *Shigella* and enteroinvasive *Escherichia coli* in human feces. Diagn. Microbiol. Infect. Dis., 28: 19-25.

Ivanova EP, Alexeeva YV, Pham DK, Wright JP, Nicolau DV (2006). ATP level variations in heterotrophic bacteria during attachment on hydrophilic and hydrophobic surfaces. Intern. Microbiol., 9: 37-46.

Labadie J, Desnier I (1992). Selection of cell wall antigens for the rapid detection of bacteria by immunological methods. J. Appl. Bacteriol., 72: 220-226.

Lee JY, Deininger RA (2004). Detection of *E. coli* in beach water within one hour using immunomagnetic separation and ATP

bioluminescence. Luminescence, 19: 31-36.
Masuko M, Kataoka T, Sugiyama N, Uchiyama S, Sugiyama H, Tarui K, Kamiya K, Kawai S (1992). A photon counting TV camera equipped with an image guide for rapid detection and counting of single bacteria in a wide field. Photochem. Photobiol., 56: 107-111.
Murphy SC, Kozlowski SM, Blander DK, Boor KJ (1998). Evaluation of adenosine triphosphate-bioluminescence hygiene monitoring for trouble-shooting fluid milk shelf-life problems. J. Food. Sci., 81: 817-820.
Murray HD, Schneider DA, Gourse RL (2003). Control of rRNA expression by small molecules is dynamic and non-redundant. Mol. Cell, 12: 125-134.
Nuzback DE, Bartley EE, Dennis SM, Nagaraja TG, Galitzer SJ, Dayton AD (1983). Relation of rumen ATP concentration to bacterial and protozoal numbers. Appl. Environ. Microbiol., 46: 533-538.
Odebrecht E, Schmidt HJ, Franco BDGM (2000). Studies on applicability of bioluminescence in the brewery. Comparative studies and critical evaluation. Brauwwelt, 140: 1904-1915.
Regnault B, Martin-Delautre S, Lejay-Collin M, Lefèvre M, Grimont PAD (2000). Oligonucleotide probe for the visualization of *Escherichia coli*/*Escherichia fergusonii* cells by *in situ* hybridation: specificity and potential applications. Res. Microbiol., 151: 521-533.
Sakakibara T, Murakami S, Eisaki N, Nakajima M, Imai K (1999). An enzymatic cycling method using pyruvate orthophosphate dikinase and firefly luciferase for the simultaneous determination of ATP and AMP (RNA). Anal. Biochem., 228: 94-101.
Sakakibara T, Murakami S, Imai K (2003). Enumeration of bacterial cell numbers by amplified firefly bioluminescence without cultivation. Anal. Biochem., 312: 48-56.
Satoh T, Kato J, Takiguchi N, Ohtake H, Kuroda A (2004). ATP amplification for ultrasensitive bioluminescence assay: detection of a single bacterial cell. Biosci. Biotechnol. Biochem., 68: 1216-1220.
Selan L, Berlutti F, Passariello C, Thaller MC, Renzini G (1992). Reliability of a bioluminescence ATP assay for detection of bacteria. J. Clin. Microbiol., 30: 1739-1742.
United States Environmental Protection Agency, Sample preservation. In: U.S.E.P.A. (1983). Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020. Cincinnati, Ohio, USA, pp. XV – XX.
Varfolomeyev SD, Gurevich KG (1999). Biokinetics (Practice Course), Fair-Press, Moscow, Russia (in Russian).
Weinrich LA, Giraldo E, LeChevallier MW (2009). Development and application of a bioluminescence-based test for assimilable organic carbon in reclaimed waters. Appl. Environ. Microbiol., 75: 7385-7390.