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# Adenosine Testing and its Interpretation in Oil and Gas Field Microbial Status

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## 1. Introduction

Microorganisms can cause multiple threats in oil and gas production (European Federation of Corrosion, 1992), injection and fracturing operations. Those threats range from production downtime due to microbial influenced corrosion (MIC) downhole and on surface equipment, to formation souring, as well as flow disruption caused by overgrowth of biomass in flow lines (European Federation of Corrosion, 1991). Adenosine analysis, particularly ATP field tests (NACE, 2014) (NACE, 2012), have been introduced into oil and gas for microbial status assessment. It facilitates a fast field screening Test for microbial status comparing to the labour intensive and time consuming culturing techniques. However, the challenge is on results interpreting. Understanding the adenosine, adenosine testing and their biological meaning are utmost important to assess the oil and gas field microbial status.

## 2. Adenosine speciation

Adenosine Triphosphate (ATP), Adenosine diphosphate (ADP), and Adenosine Monophosphate (AMP) in the living cells are involving with energy regeneration cycle. All are playing important role in cellular energetic metabolism cycle.

**ATP: Adenosine triphosphate**

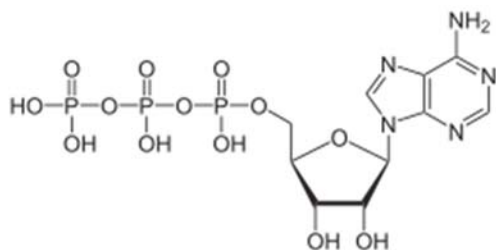


Figure 1 Molecular structure of ATP  
([https://en.wikipedia.org/wiki/Adenosine\\_triphosphate](https://en.wikipedia.org/wiki/Adenosine_triphosphate), n.d.)

**ADP: Adenosine diphosphate**

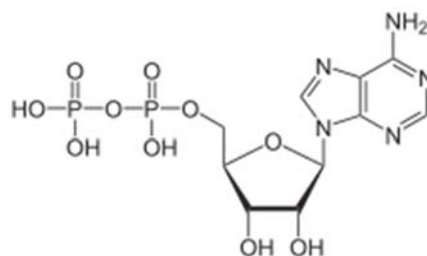


Figure 2 Molecular structure of ADP  
([https://en.wikipedia.org/wiki/Adenosine\\_diphosphate](https://en.wikipedia.org/wiki/Adenosine_diphosphate), n.d.)

**AMP: Adenosine monophosphate**

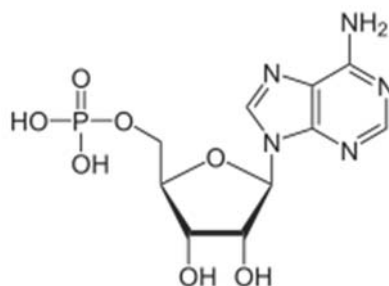
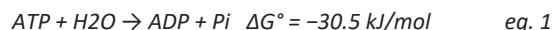


Figure 3 Molecular structure of AMP  
([https://en.wikipedia.org/wiki/Adenosine\\_monophosphate](https://en.wikipedia.org/wiki/Adenosine_monophosphate), n.d.)

ATP is hydrolysed to release energy and produce ADP and AMP, while AMP and ADP can be converted back to ATP utilizing the energy in food. These energetic metabolism reactions are indicated as:

Using ATP as metabolism energy source:





Storing energy in ATP by converting AMP and ADP to ATP:

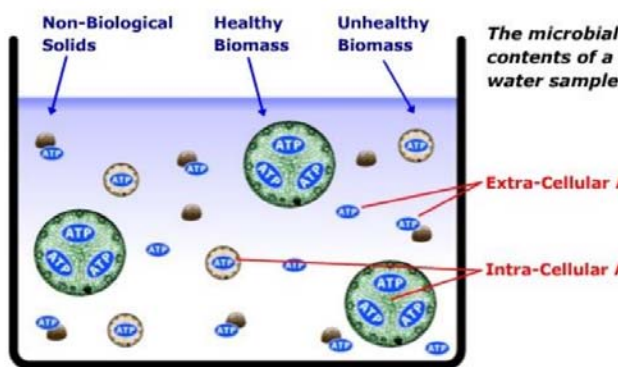
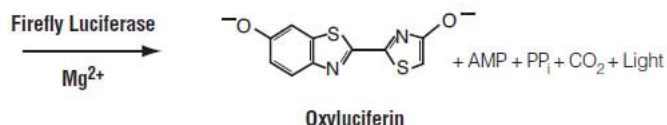
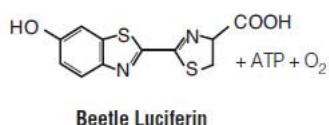


Figure 4 illustration of intra-cellular ATP and extra-cellular ATP

Adopted from Luminultra website (<https://www.luminultra.com/tech/>)

ATP is not only present within the living cell, i.e. intracellular ATP, it is also released into surrounding media by living, stressed as well as dead cell, i.e. extracellular ATP.

The microbial status assessment should consider all these sources of the ATP before analytical results can be interpreted meaningfully.



eq. 5 Luciferase reaction to quantify the ATP

### ATP analysis

ATP is the most common substance to be tested. Since ATP exists both inside (intra-cellular ATP) and outside the cell (extra-cellular ATP), information for extra-cellular ATP and intra-cellular ATP is required for the meaningful interpretation. Some analytical procedure may involve a filtration step to separate the intra-cellular and extra-cellular ATP

For ATP determinations, sample mixes with  $MgSO_4$  and Tris/acetic acid at pH 7.3. ATP then reacts with luciferin. In this reaction, luciferin is oxidized to oxyluciferin with the 560nm light emission in the presence of  $Mg^{2+}$  and firefly luciferase as catalyst. When ATP is the limiting component in this luciferase reaction, the intensity of the emitted light is proportional to ATP concentration. Measurement of the light intensity using a luminometer permits direct quantitation of ATP (Promega, 2015)). The following formula below shows the reaction that occurs during the ATP analysis:

### 3. Adenosine testing

Even most of adenosine testing is referring to ATP testing in oil and gas industrial microbial assessment, ADP and AMP also have significance in microbial status assessment. The most common available analytical procedures for adenosine analysis are briefly discussed here:

### ADP analysis process

There is a ADP analytical method marketed as Promega ADP-Glo® (Promega, 2015)), in which a two-step process is utilised to analysis the ADP within the system. In this process, the pre-existing ATP is removed before ADP is converted to ATP, the final ATP is analysed by luciferase reaction as discussed in eq. 5, which is stichometric to the ADP in the analyte.

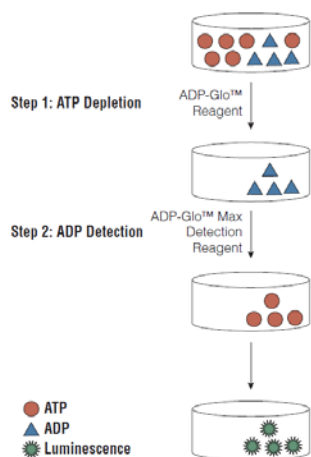


Figure 5 ADP analysis process by Promega ADP-Glo®

In step 1, ADP-Glo™ Reagent is added to terminate the enzymatic reaction and to deplete the remaining ATP, and in step 2, the ADP-Glo™ Max Detection Reagent is added to convert ADP to ATP and allows the newly synthesized ATP to be measured using a luciferase/luciferin reaction (eq. 5). The light generated correlates with the amount of ADP in the quenched system.

### AMP analysis process

There is a AMP determination method using Promega AMP-Glo® (Promega, 2015)), in which a two-step process is utilised to analysis the AMP within the system. In this process, the pre-existing ATP is removed before AMP converts to ADP. ADP is then converted to ATP and determined through luciferase reaction (eq. 5). The amount of AMP in the system is stoichiometrically through final ATP measured (Figure 6) .



Figure 6 AMP analysis process by Promega AMP-Glo®

### ATP + ADP analysis

It was reported that the sum of ATP plus ADP can be analysed using luciferase reaction (eq. 5). All ADP are stoichiometrically converted to ATP before ATP is quantified through the luciferase reaction. The sample mixes with MgSO<sub>4</sub> and Tris/acetic acid at pH 7.3 containing phosphoenolpyruvate and pyruvate kinase (EC. 2.7. 1.40). These converts all ADP to ATP before it reacts with luciferin in the presence of luciferase to obtain the total amount of ATP and ADP (Niven, Collins, & Knowles, 1977).

### ATP+ADP+AMP analysis

For total adenylate determinations, sample mixes with MgSO<sub>4</sub> and Tris/acetic acid at pH 7.3, it further reacts with phosphoenolpyruvate, pyruvate kinase and adenylate kinase (EC. 2.7.4.3). These mixtures were incubated at 30 C for 15 min and held on ice until assayed (< 15 min). The total ATP is the sum of pre-existing ATP, ADP and AMP (Niven, Collins, & Knowles, 1977)

## 4. Significance of ATP/ADP/AMP in microbial activity assessment

ATP, ADP and AMP in the living cells are involving with energy regeneration cycle. ATP is hydrolysed to release energy and produce ADP and AMP, while AMP and ADP can be converted back to ATP utilizing

### Adenosine analytical methods accuracy

1) Since the adenosine test measures Relative Light Units (RLU's) as the direct output, which is proportional to the analyte level. This result can be impacted by not only the quantity of ATP in the sample, but also the reaction temperature, the luminometer make, model and overall

condition, the enzyme age, potency and concentration, among other things.

- 2) Most extra-cellular ATP is complexed to various molecules and cell debris, and is unavailable participate in luciferase reaction (eq. 5). When intra-cellular ATP is extracted, it readily complexes or degrades, making it unavailable to luciferase reaction (eq. 5) as well. Therefore, it is important to ensure that all complexed extra-cellular ATP and all extracted intra-cellular ATP are analyzed in sample. For this reason, ATP testing has only limited success in water, wastewater and oil and gas industrial applications so far.
- 3) Samples often contain components that inhibit or quench the bioluminescent luciferase reaction. These either enhance or inhibit the luminescence reaction. Considering the complicate sampling matrixes, e.g. sample colour, dissolved solids, suspended solids organics, heavy metals, biocides and organic solvents etc, it is important that the effects of all of these various components that may be present in a sample are minimized or neutralized prior to the luciferase reaction (eq. 5) (Luminultra microbial monitoring);

### The impact of living microorganism on analytical accuracy of adenosine

The constant conversion of ATP/ADP and AMP in living cells, which suggested the particular components of the interest need to be quenched during the analysis to indicate a meaningful snapshot of microbial activities. ATP is unstable and readily hydrolyses to ADP and AMP, stabilizing all released intra-cellular ATP and as well as extra-cellular ATP prior to assaying to prevent its degradation between recovery and assay.

### ATP in microbial status assessment

ATP is taken as microbial energy token for cellular metabolism. Higher ATP is usually being regarded as more active bioactivities. although the criterion is generally valid in microbial assessment, its limitations are also obvious:

- 1) It is difficult to quantify the bioactivities through ATP due to the coexistence of intra-cellular and extra-cellular ATP;
- 2) It is difficult to quantify the specific microorganism may cause the harmful Microbiologically Influenced Corrosion (MIC) in oil and gas industry due to the non-specific nature of ATP. ATP / ADP and AMP are not specific to particular organisms, therefore, their level is the overall microbial activities rather than the specific microorganism of the concern.
- 3) It is difficult to assess the growth stage (corrosion risks) of microorganism by ATP.
- 4) Particular attention to contamination during sampling , storage, transferring and analytical process.

### Adenosine Energy Charge (AEC) in microbial status assessment

AEC is defined as  $([ATP + 1/2ADP]/[ATP + ADP + AMP])$ . Adenylate energy charge(AEC) remains relatively constant during the growth stage and early stationary phase of the growing cycle, while the intracellular ATP amount increased with biomass. It is generally believed that starvation results in lower AEC. It is usually accepted that the AEC would be between 0.8~0.9 in healthy microorganism, in which actively growing and dividing cells are normally identified. AEC below 0.8 suggested the sub-optimal condition. Some suggested cells in stationary growth phase maintains a AEC of 0.6. While AEC decreased below 0.5, the colony is stressed, a senescent or resting phase cells are normally having this AEC value. The growth and

reproductive rates are much reduced while AEC is below 0.8. ( WIEBE & BANCROFT)

### ATP/AMP ratio

Nalco and Champion's AccuCount® system using ATP and AMP ratio to estimate the quantities of active and dormant microorganism in the system. It also uses a two-step process to determine the ATP and AMP amount in the sample through luciferase reaction (eq. 5). They claim, by using ATP and AMP, having improved understanding of active bacteria area and effectiveness of chemical treatment (NALCO Champion).

### Comparing of adenosine testing with culturing techniques in microbial status assessment

An average *E. coli*-sized bacteria cell will contain approximately 1 femtogram (fg) of ATP, while the average fungus contains 10 to 100 fg ATP/mL. It is reported that visible colony is  $\sim 10^9$  cells (Luminultra microbial monitoring, 2016). Utilizing this information, we can estimate CFU/mL (www.luminultra.com, n.d.) However, there is no current information on ATP/ADP/AMP level on bacteria speciation particularly of the interest for oil and gas industry.

## 5. Conclusion

Adenosine test provide a fast, efficient screening tool for oil and gas microbial status assessment. It is important for Corrosion engineer to understand the principle and limitation of this tool, as well as its difference to traditional culturing techniques. It is an on-going challenge to monitor the microbial status and validate the adenosine testing results with other field culturing techniques.

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