# Summary of Findings

## Wastewater Treatment Simulation using BIOLOGIC SR2 from SciCorp

## Introduction

BIOLOGIC SR2 from SciCorp is a concentrated bio-stimulant that is intended to increase microbial growth, improve sludge quality and settling, increase cellular metabolism, and suppress $H_2S$ generation by reduction of $SO_4$. The goal of this experiment was to assess its effectiveness to stimulate growth of new, healthy aerobic biomass using influent and sludge from a municipal wastewater treatment plant. In addition, it focused on the ability of LuminUltra's QuenchGone21 Wastewater test protocol to demonstrate the metabolic benefits of this product as a wastewater bioadditive.

## Experimental Setup

The experiment focused on simulating aerobic and anoxic conditions using various methods and compared biomass response with and without *BIOLOGIC SR2* added, as shown below:

1. Aerobic (Aeration Stones) - Add 500mL of Raw BWWT Influent and 88mL of MLSS to four (4) aerated vessels. Prepare the reactors as follows:
    a. Straight aeration (Control)
    b. *BIOLOGIC SR2* (Normal Dosage) – add 6.25µL of *BIOLOGIC SR2* to produce a 12.5mg/L dosage.
    c. *BIOLOGIC SR2* (1/2 Normal Dosage) – add 3.125µL of *BIOLOGIC SR2*, equivalent to half the normal dosage of *BIOLOGIC SR2*.
    d. *BIOLOGIC SR2* (2x Normal Dosage) – add 12.5µL of *BIOLOGIC SR2*, equivalent to twice the normal dosage of *BIOLOGIC SR2*.
2. Anoxic (Lab Stirrer) - Add 500mL of Raw BWWT Influent and 88mL of MLSS to two (2) 1L covered polypropylene jars and set up on the laboratory stirrer. Prepare the reactors as follows:

a. Anoxic (control)
b. *BIOLOGIC SR2* (Normal Dosage) – add 6.25µL of *BIOLOGIC SR2*.

The sludge volumes were chosen based on the F/M ratio and SRT of the wastewater treatment process from which they were taken.

## Daily Maintenance

1. Using LuminUltra's QuenchGone21 Wastewater (QG21W) test kit, each vessel was tested daily for Total ATP (tATP), Dissolved ATP (dATP), Planktonic ATP (pATP) and Floc Bulking ATP (fbATP). From these results, the following quantities were calculated:
   - Cellular ATP (cATP) – represents the ATP associated with living cells only and is a direct indicator of the potential of the biomass population to degrade waste.
   - Biomass Stress Index (BSI) – represents the ratio of dATP to tATP and provides a indication of the stress level that the population is under due to the conditions of its environment.
   - Planktonic ATP (pATP) – measures the percentage of biomass that does not settle (due to deflocculation).
   - Floc Bulking ATP (fbATP) – measures the percentage of the biomass population made up of filamentous bacteria.
2. Nutrient top-up – on a daily basis, each bioreactor was dosed with the following nutrients at a 100C : 5N :1P ratio to ensure that the biomass was not nutrient-deprived:
   - $C_6H_{12}O_5$ @ 2000mg/L (800 mg/L C)
   - $NH_4Cl$ @ 153mg/L (40 mg/L N)
   - $Na_3PO_4$ ·12 H2O @ 98mg/L (8 mg/L P)
3. pH Adjustment – to ensure that all bioreactors operated under the same pH conditions, they were monitored and adjusted twice daily to maintain a neutral pH (7) using 2% $H_2SO_4$ and 1% NaOH as necessary.
4. DO Adjustment – the dissolved oxygen concentration in the aerated vessels were measured, recorded, and adjusted to maintain 2.0mg/L on a daily basis.

# Results

<u>Biomass Growth Trends</u>

The main goal of the simulation was to observe the living biomass' response to the addition of SciCorp's Biologic SR2 additive in various dosages by trending the Cellular ATP (cATP) in each vessel. Rather than relying on TSS or VSS measurements that contain many components other than active biomass, monitoring the cATP of a bioreactor isolates the living population and subsequently allows the user to observe its response to process changes.

By trending the cATP of the aerated vessels, the benefit of the Biologic SR2 additive was clearly seen via the significant growth that occurred early on in the experiment. By Day 2, the Normal Dosage and ½ Dosage scenarios experienced significantly elevated growth while the Control showed a slight decline in the active biomass population. However, the 2x Dosage appeared to adversely affect the biomass.

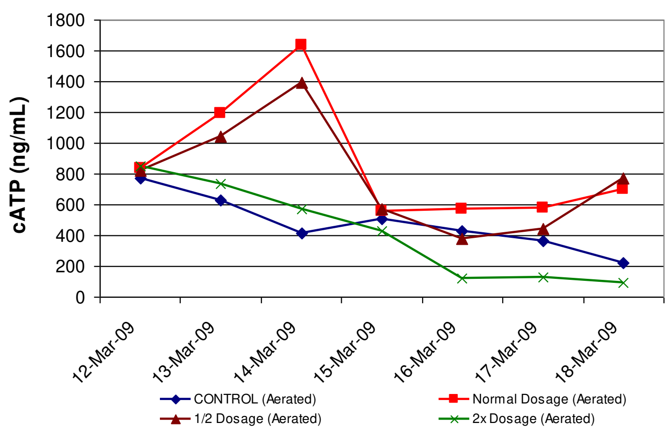


**Figure 1: Biological Content for Each Scenario (7-Day Averages)**

The trend of early growth followed by a "crash" period and eventual modest rebound is consistent with what has been seen in long-term laboratory incubations done in the past. Since it is nearly impossible to match real-world conditions (i.e. hydraulics, sludge wasting & return, etc.), this behavior is likely a product of the limitations of the test set-up.

When the results are normalized to the Control, the benefits of Biologic SR2 are unmistakable. After only two days, the reactor with the recommended dosage reached an active biomass population that was 4 times greater than that of the control.

While the magnitude was smaller, a similar increase was seen with the ½ dosage, indicating that even a small amount provides significant benefit. While the 2x dosage shows a minimal improvement over the Control, it is clear that under-dosing is preferred to over-dosing.

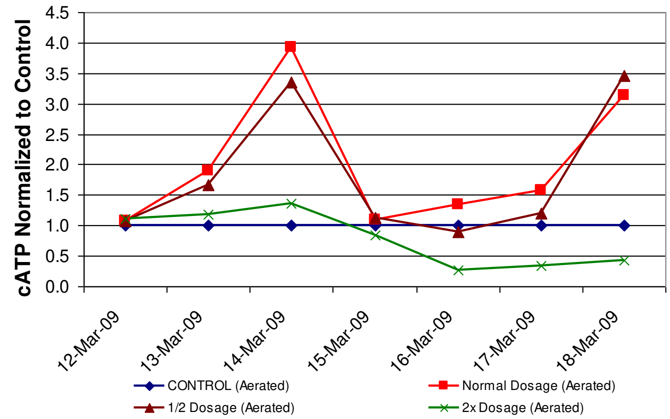


**Figure 2: Aerobic Growth Trend Normalized to Control**

The Mixed Liquor used in the simulation was from a conventional aerated activated sludge reactor, so it is not surprising to see that the population showed a decreasing trend under anoxic conditions. Since the indigenous biomass was acclimatized to an oxygen-rich environment, the population quickly declined when incubated under anoxic conditions.

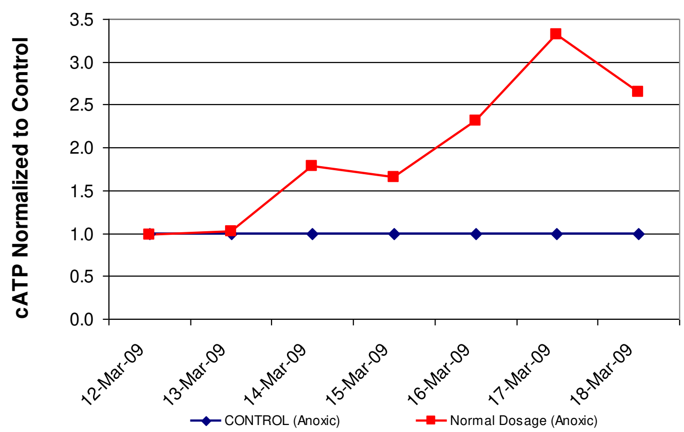


**Figure 3: Anoxic Growth Trend Normalized to Control**

Despite the drop in biological activity under anoxic conditions, it is clear that the addition of Biologic SR2 had a positive impact on the population. While the dosed vessel experienced the same declining trend, it was not nearly as severe. When the results normalized to the control, the benefit of the Biologic SR2 addition is even more clear, despite the unfavorable conditions. Since the bio-additive decreased the rate that the population dropped in the absence of oxygen, it could potentially soften the effects of loss of aeration in a treatment plant.

## Solids Viability

If the Total Suspended Solids (TSS) of a bioreactor sample is known, cATP results can be converted to a mass basis and normalized to the TSS to determine the Active Biomass Ratio (ABR). The ABR represents how much of the bioreactor's solids inventory is made up of living biomass. Maximizing the ABR is always in the best interest of plant operators since it means that excess useless solids (dead biomass, inert material, etc.) is kept to a minimum. A high ABR value often results in better floc quality, improved settling, and better overall plant efficiency.

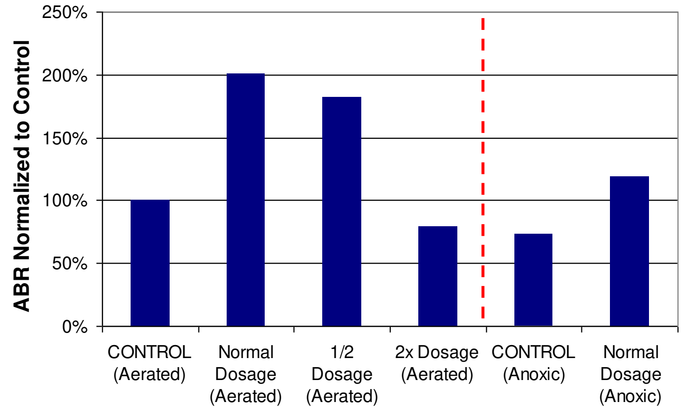


**Figure 4: Effects of Biologic SR2 on Solids Viability**

Since cATP results from each scenario has established that Biologic SR2 promotes enhanced, growth, a dosing program using Biologic SR2 has the potential to improve the ABR and subsequently improving plant efficiency. Depending on the goals of the plant, ABR optimization can be used in the following ways:

1. To Increase Plant Capacity – Bioreactors that are running at or near their capacity are often overloaded with solids. In many cases, this is due to too much solids being returned to the process, resulting in a large amount of useless solids being concentrated in the bioreactor. In these situations, plant capacity is often limited by the rate at which secondary clarification can remove these solids downstream of the reactor.

   By routinely monitoring the ABR in the bioreactor, carefully adjusting sludge waste rates, and dosing with Biologic SR2 accordingly, the active biomass population (as measured using cATP) can be increased while keeping the total solids inventory the same (or possibly decreasing it). This is achieved by slowly purging excess solids from the process while ensuring that a sufficiently large active population remains.

2. To Minimize Costs – Solids handling (pumping, dewatering, landfilling) accounts for a very large percentage of the costs associated with wastewater treatment. Therefore, by using a similar strategy as outlined above, the total solids inventory can be decreased while maintaining a sufficiently large population to treat plant influent in order to operate with a lower solids inventory. Carefully waste a slightly higher than normal amount of sludge for a short time, then promote growth of a healthier population by using a bio-stimulant such as Biologic SR2. This slowly decreases the amount of accumulated solids in the system and replaces them with living healthy biomass, thus decreasing the improving the solids viability and increasing the capacity of the plant to treat waste.

## Stress Assessment

The Biomass Stress Index (BSI) parameter indicates the relative stress that the biomass population is experiencing at the point in the process from which the sample was drawn. When used as part of a routine monitoring program in a wastewater treatment process, it enables the user to quickly identify stressful conditions (i.e. influent toxicity) and troubleshoot the situation before severe problems results.

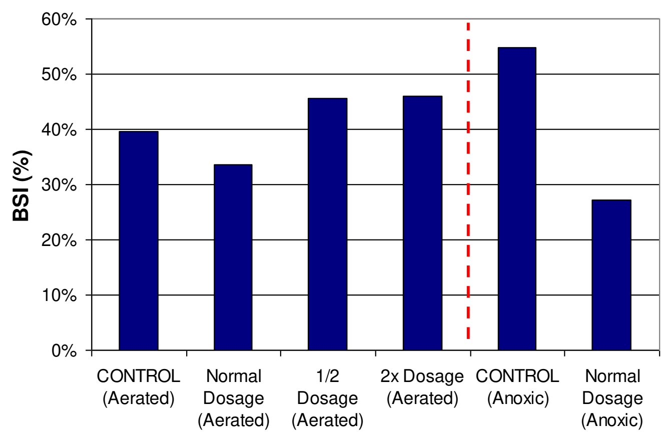


**Figure 5: Average BSI Values for Each Scenario**

In this situation, it is not surprising that SR2 did not reduce stress in the aerated vessels since all of the reactors were treating the same substrate. However, it was somewhat surprising to see that the BSI was significantly lower in the dosed anoxic vessel than in the Anoxic Control.

## Assessment of Floc Physiology

Under aerobic conditions, Biologic SR2 appears to 'thicken' sludge by increasing fbATP and reducing small floc particles. If kept in check this would be highly beneficial to ensure good floc structure and to sustain good settling performance. However, elevated fbATP levels above baseline can also be an early indicator of the proliferation of filamentous bacteria. Therefore, this protocol can also provide an early warning of a sludge bulking event. Interestingly enough, it appears that the additive has the opposite effect under anoxic conditions.

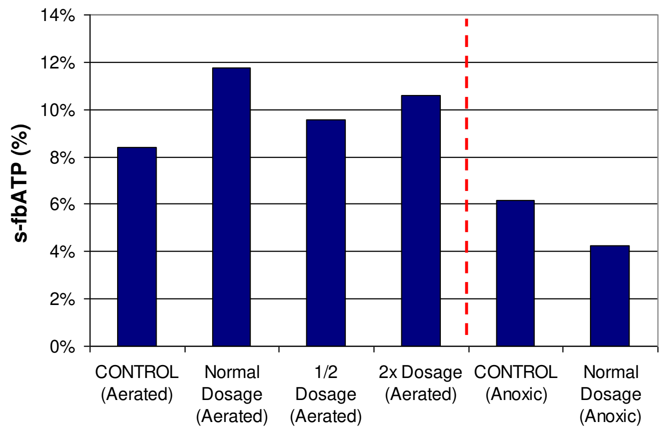


**Figure 6: Average fbATP Values for Each Scenario**

The final routine test involved assessing the degree of deflocculation that was taking place in each reactor. One interesting observation from these results was that the Anoxic scenarios showed higher pATP levels than the Aerobic vessels. Since previous results showed that the population significantly decreased in the Anoxic vessels, it seems logical that the pATP would be higher (biomass spreads out) because there was more food available for the smaller population. High F/M ratios often result in deflocculation, causing elevated pATP levels.

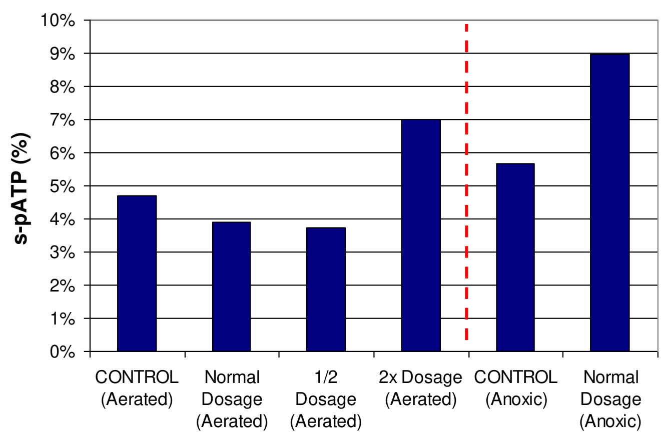


**Figure 7: Average fbATP Values for Each Scenario**

## Conclusions

At the conclusion of the experiment, the results clearly showed that, when used properly, Biologic SR2 from SciCorp can provide the following to the operation of wastewater treatment plants (WWTPs):

- Increased metabolic activity of living biomass under all conditions;
- Reduced biological stress and pin-floc formation from low DO conditions;
- Improved floc structure (thicker), which can improve settling
- Overall, improved plant performance and reduced operating costs (electrical, chemicals, sludge management).

### *Future Work*

*In the future, bench-scale tests similar to this one could be done to assist with the development of a control program for pacing bioadditive use in full-scale process situations, leading to continuous process improvement.*