Evaluation of *Spirulina platensis* in Bicarbonate-Based Integrated Carbon Capture and Algae Production System Utilizing Different Culture Media

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A method known as Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS), is a growing study introduced as an alternative to current carbon capture and sequestration (CCS) methods. It is a closed-loop cycle involving inorganic carbon in the form of bicarbonates, which is consumed by microalgae for growth and utilizes the regenerated carbonates for another cycle of carbon capture. Existing literature requires more in-depth experimentation and analysis with regards to the viability of different microorganisms to the rising method. *Spirulina platensis* was evaluated in BICCAPS using 0.1M Na$_2$CO$_3$, employing three different culture media for growth, namely, modified Zarrouk's, NPK-based, and NPK-based with A$_5$ solution media. Biomass growth, productivity, and carbon dioxide utilization were investigated to determine the effectivity of BICCAPS as a carbon sequestration technique. At control conditions, NPK-based with A$_5$ solution medium yielded the highest productivity with a value of 10.81 mg L$^{-1}$ day$^{-1}$. Likewise, using NaHCO$_3$ as a carbon source, results show that the highest productivity was achieved also under NPK-based with A$_5$ solution medium with a value of 6.80 mg L$^{-1}$ day$^{-1}$, as well as a high carbon conversion value of 2.092 day$^{-1}$.

**Keywords:** BICCAPS, Carbon Capture, Modified Zarrouk’s Medium, NPK-based Medium, Sodium Bicarbonate, *Spirulina platensis*

**INTRODUCTION**

Combustion or burning of fossil fuels has become a well-known and accepted method in energy production (Sayre 2010). Actuated by the demands during the industrial revolution and with the current rapid economic growth, CO$_2$ content in the atmosphere has drastically increased, resulting to global warming (Leung, Caramanna, & Maroto-Valer, 2014). According to the International Energy Agency (IEA), in 2018 alone, atmospheric CO$_2$ drastically increased by...
1.7% at a peak value of 33.1 gigatonnes or 407.4 ppm. IEA further assessed that from the 1°C increase in global average surface temperature, 0.3°C of which is caused solely by production of the fossil fuels. As a result, coal has been identified as the most significant lone contributor to temperature rise (IEA 2019).

Carbon Capture and Sequestration (CCS) is a method addressing global warming through CO₂ reduction by capturing, transporting, and storing CO₂ from industrial or utility plants in deep aquifer formations (CCSA 2011, Herzog and Golomb 2004, MIT 2004). However, current carbon capture methods were found disadvantageous since it generates other gaseous wastes, and requires high cost from chosen capture method and transportation (Chi et al. 2013a).

Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS) is a newly-proposed carbon capture method. This method effectively satisfies the need to capture CO₂, has lower costs from sustainable energy, provides large amounts of biomass as feedstock to valuable products. This technology applies the closed-loop process, involving the formation of bicarbonate from carbon dioxide capture using carbonate, microalgae’s utilization of bicarbonate as carbon source, and regeneration of carbonate from bicarbonate’s consumption (Chi et al. 2013b).

The microalgae must be able to withstand high pH values and encompass high carbon fixation rates to survive BICCAPS; additionally, this considers and prefers high biomass productivities in selecting microalgal candidates (Chi et al., 2013a). Singh and Singh (2014) compares microorganisms in terms of CO₂ fixation rate as well as biomass productivities upon loading a certain %CO₂; the best results were identified with: Synechocystis aquatilis, Botryococcus barausni, Chlorella vulgaris, Spirulina platensis, and Dunaliella tertiolecta. Considering only alkaliophilic microalgae, only Spirulina platensis and Dunaliella tertiolecta were qualified candidates. Although Chlorella vulgaris is a freshwater microalga, it is known to survive harsh conditions. In this regard, present literature indicates that BICCAPS has been applied to Chlorella vulgaris (Mokashi et al. 2016) and Dunaliella salina (Kim et al. 2017).

Spirulina platensis was selected for the study, considering the availability of certain microalgae in tropical countries like the Philippines, as well as the scarcity of literature present.

Previous studies have proven the ability of S. platensis to grow with CO₂ or HCO₃⁻ as a carbon source under various media. Sydney et al. (2010) conducted a study wherein air, with 5% CO₂ sparged through S. platensis under Zarrouk’s medium, produced maximum cell productivity of 0.73 g L⁻¹ day⁻¹. Likewise, Kumari, Kumar, Pathak, & Guria (2014) utilized a cost-effective NPK-based medium, generating maximum cell productivity of 0.42 g L⁻¹ day⁻¹.

This study evaluates the viability of Spirulina platensis in BICCAPS for CO₂ mitigation and utilization. Specifically, it determines and differentiates the growth curve, biomass productivity, maximum specific growth rate, and inorganic carbon
conversion of *Spirulina platensis* under Modified Zarrouk’s, NPK-based fertilizer, and NPK with added A5 solution culture media at controlled CO2 concentration and under BICCAPS utilizing NaHCO3 as carbon source.

**METHODOLOGY**

**Culture Media**

*Spirulina platensis* was obtained from the Southeast Asian Fisheries Development Center (Manila, Philippines) and grown under three different culture media: NPK-based, NPK-based with A5 solution, and Modified Zarrouk’s culture media – in separate containers. Modification of the original Zarrouk’s formula with regards to the removal of the NaHCO3 was done to investigate the effect of NaHCO3 in BICCAPS.

The Modified Zarrouk’s culture medium consisted of 2.5 g-L⁻¹ NaNO3, 0.5 g-L⁻¹ K2HPO4, 1 g-L⁻¹ K2SO4, 1.0 g-L⁻¹ NaCl, 0.04 g-L⁻¹ CaCl₂•2H₂O, 0.01 g-L⁻¹ FeSO₄•7H₂O, and 1 mL of A5 solution. The A5 trace elements solution is composed of 2.86 g-L⁻¹ H3BO3, 1.81 g-L⁻¹ MnCl₂ • 4H2O, 0.222 0.01 g-L⁻¹ ZnSO4 • 4H2O, 0.018 0.01 g-L⁻¹ Na2MoO4, and 0.079 0.01 g-L⁻¹ CuSO4 • 5H2O, which was based on the formulation provided by Zarrouk (1966).

NPK-based culture medium, on the other hand, was composed of 0.076 g-L⁻¹ NPK, 1 g-L⁻¹ NaCl, 0.2 g-L⁻¹ MgSO4, 0.08 g-L⁻¹ EDTA, 0.04 g-L⁻¹ CaCl₂, 0.01 g-L⁻¹ FeSO₄, 0.0207 g-L⁻¹ urea, and 0.0469 g-L⁻¹ silica, which was based on the formulation provided by Kumari, Kumar, et al. (2014), chemicals and reagents were obtained from Belman Laboratories (Philippines) and Yana Commodities (Philippines).

**Experiment**

**Cultivation with Continuous Aeration**

All experimental runs for the cultivation of the microalga involved a working volume of 8L using PET bottles at a pH of approximately 9, in 12-hour light-12-hour dark cycle with the use of a tubular LED lamp at ambient temperature. Air pumps attached with a sparger were used to continuously introduce CO2 from air at an average volume rate of 0.8 L min⁻¹. Samples for dry weight and optical density were taken thrice a week until a stationary phase was achieved. Calibration curves were generated by relating optical density with dry weight values. Biomass productivity, maximum specific growth rate, and pH for each culture media were also evaluated.

**Carbonate Dissolution and Carbon Dioxide Absorption**

Under the closed system, water was reacted with CO2 at a loading of 1 L min⁻¹ to form carbonic acid solution as seen in Eq. (1). The solution then dissociates to form bicarbonates as displayed in the reaction in Eq. (2).

\[
\text{CO}_2(\text{g}) + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3(aq) \quad (1)
\]

\[
\text{H}_2\text{CO}_3(aq) \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad (2)
\]

Sodium carbonate dissolution was performed, producing 0.1M Na2CO3 solution; except for the control system where no carbonate was introduced. This solution was subjected to CO2 bubbling to produce sodium bicarbonate solution as can be seen in Eq. (3).

\[
\text{H}_2\text{CO}_3(aq) + \text{Na}_2\text{CO}_3(\text{s}) \rightarrow \text{Na}_2\text{CO}_3(aq) + 2\text{HCO}_3^- \quad (3)
\]
**Cultivation in BICCAPS**

Resulting bicarbonate solutions were utilized in a closed system cultivation of *Spirulina platensis*. The microalgal specie was cultivated under modified Zarrouk’s, NPK-based, and NPK with A5 solution culture medium in tightly sealed PET bottles at a working volume of 5L for approximately 30 days or until the stationary phase was observed. During cultivation period, samples were obtained thrice a week to evaluate its optical density, carbonate, bicarbonate, and dissolved CO₂ content. Nomenclature list of these systems are listed in Table 1.

### Table 1. Nomenclature list for the cultivation of *Spirulina platensis*

<table>
<thead>
<tr>
<th>Culture Media</th>
<th>Carbon Source</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified</td>
<td>Air CO₂(g)</td>
<td>ZO</td>
</tr>
<tr>
<td>Zarrouk’s</td>
<td>Na₂CO₃ bubbled with CO₂(g)</td>
<td>ZS</td>
</tr>
<tr>
<td>NPK-based</td>
<td>Air CO₂(g)</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>Na₂CO₃ bubbled with CO₂(g)</td>
<td>NS</td>
</tr>
<tr>
<td>NPK with A5</td>
<td>Air CO₂(g)</td>
<td>AO</td>
</tr>
</tbody>
</table>

**Analytical Method**

**Cell Growth Measurement**

Biomass was obtained by applying vacuum filtration. As seen in Eq. (4), biomass productivity is obtained from the difference between final and initial concentrations found in the exponential phase. To evaluate the maximum specific growth rate of the *S. platensis* in the culture media, Eq. (5) is used, which displays the linearized relationship of biomass concentration against time.

\[ \text{Biomass productivity} = \frac{X - X_0}{t} \]  
\[ \ln( X ) = \mu_{\text{max}}t + \ln( X_0 ) \]  

**Optical Density**

The optical density was measured at a wavelength of 530 nm using a Shimadzu UV-1700 spectrophotometer (Japan) in the duplicate analysis. The Beer-Lambert’s Law is applied, as seen in Eq. (6).

\[ A = \log_{10} \frac{1}{T} = \log_{10} \left( \frac{I_0}{I} \right) A = \varepsilon c d \]  

**Carbonate and Bicarbonate Concentration**

Carbonate and bicarbonate concentrations were regularly evaluated using a double titration technique for a 5-mL filtered sample. The amount of the carbonate can be determined by titrating the sample with 0.1 M HCl using phenolphthalein indicator. A second titration was performed using a bromocresol green indicator with the same acid to determine the amount of bicarbonate in the solution. The following reactions, as presented in Eq. (7) and (8) were observed until the endpoint for each titration analysis was achieved.

\[ \text{CO}_3^{2-} + \text{H}^+ \rightarrow \text{HCO}_3^- \]  
\[ \text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{CO}_3 \]  

**Dissolved CO₂ Concentration**

A 5-mL filtered sample was added with an excess volume of 0.1 M NaOH. This
reaction allows the conversion of dissolved carbon dioxide into sodium carbonate, as seen in Eq. (9). Excess 1 M BaCl₂ sample was added to precipitate carbonates found in the solution, forming a white-opaque solution as expressed in Eq. (10). The unreacted NaOH is back titrated with 0.1M HCl solution to a phenolphthalein endpoint, as shown in Eq. (11).

\[
\text{CO}_2 + 2\text{NaOH} \rightarrow \text{Na}_2\text{CO}_3 + \text{H}_2\text{O} \quad (9)
\]

\[
\text{Na}_2\text{CO}_3 + \text{BaCl}_2 \rightarrow 2\text{NaCl} + \text{BaCO}_3 \quad (10)
\]

\[
\text{NaHCO}_3 + \text{NaOH} \rightarrow \text{2NaCl} + \text{H}_2\text{O} \quad (11)
\]

It can be determined that the amount of NaOH reacted in the solution is the total amount of CO₂ and bicarbonate. Hence, to calculate for dissolved CO₂ content (nₗ₅ dissolved CO₂) in the sample, moles of bicarbonate (nₗ₅ HCO₃⁻) reacted is subtracted from moles of CO₂ (nₗ₅ CO₂) that reacted with NaOH. Eq. (12) is applied to solve for moles dissolved CO₂.

\[
n_{\text{dissolved CO}_2} = \frac{n_{\text{CO}_2\text{ reacted}}}{2} - \frac{n_{\text{HCO}_3^-\text{ reacted}}}{2} \quad (12)
\]

**Inorganic Carbon Conversion**

The amount of CO₂ converted into biomass per volume of culture per day is termed as inorganic carbon (Cᵢ) conversion, as shown in Eq. (13). In BICCAPS, total amount of CO₂ utilized includes the quantity of carbonate produced after cultivation, as well as quantity of dissolved CO₂ consumed in the culture medium, as seen in Eq. (14).

\[
\text{Ci conversion} = \frac{\Delta \text{Ci}}{\text{culture time}} \quad (13)
\]

\[
\Delta \text{Ci} = [\text{CO}_3^{2-}]_{\text{prod}} + [\text{d. CO}_2]_{\text{consumed}} \quad (14)
\]

**RESULTS AND DISCUSSION**

**Spirulina Platensis Cultivation**

Cultivation of microalgae *Spirulina platensis* was regularly analyzed and monitored to maintain proper operating conditions. The average of the calibration curves generated for each culture media is found in the supplementary information, with linearity values of 0.7817, 0.8441, and 0.8935 for Modified Zarrouk’s, NPK-based, and NPK with A₅ solution, respectively. The resulting linear equation from each calibration curve was used to calculate biomass concentration from absorbance value for BICCAPS.

Figure 1 displays the successful growth of *S. platensis* under modified Zarrouk’s media, NPK-based, and NPK-based with A₅ solution media. This is further characterized using a maximum specific growth rate (μₘₐₓ) by relating the natural logarithm of dry weight with time.

A summary of obtained biomass productivities, as well as maximum specific growth rate values, are tabulated in Table 2.
Table 2. Summary of Values Obtained Under Open System

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ZO</th>
<th>NO</th>
<th>AO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass productivity</td>
<td>11.19</td>
<td>11.00</td>
<td>22.08</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>0.0469</td>
<td>0.0400</td>
<td>0.0875</td>
</tr>
</tbody>
</table>

From this, it can be seen that *S. Platensis* cultivated in NPK with A5 obtained the highest biomass productivity, followed by Modified Zarrouk’s and closely succeeded by NPK-based. ZO system was expected to embody the highest biomass productivity since Zarrouk’s medium was formulated specifically for optimal *Spirulina platensis* growth. However, due to the removal of NaHCO3, Na+ was suggested to be insufficient for ideal biomass growth, as supported by studies of Kumari et al. (2014). Also, it has been previously established that NPK medium produces relatively lower productivities as compared to Modified Zarrouk’s; however, with the addition of A5 micronutrients, the biomass productivity of AO was seen to have doubled as compared to that of NO. Upon subjecting this to one-way ANOVA, the effect of varying culture media with biomass productivity under an open system is significant ($p<0.05$).

**Carbon Dioxide Absorption of Control System and Sodium Carbonate Salt System**

The behavior of CO$_3^{2-}$, HCO$_3^-$, and d. CO$_2$ concentrations were observed under the control system and illustrated in Figure 2a. Bicarbonate concentration increased to a maximum concentration of 5.69 mM at the 5th minute and continued to decrease after producing a new equilibrium system. Dissolved CO$_2$ concentration, however, increased and peaked at the 15th minute with a concentration of 9.70 mM.

For the sodium carbonate solution, CO$_2$ absorption was successful as the number of bicarbonates increased twice as much like that of carbonates after 150 minutes. It can be seen from figure 2b, with the initial concentration of carbonate 280.05 mM and the final concentration of bicarbonate 335.88 mM, the CO$_2$ absorption lead to a yield of 74.61%. Furthermore, a CO$_2$ absorption ratio of 1.4496 was obtained, corresponding to moles of CO$_2$ absorbed per mole of carbonate consumed.

![Figure 2a](image1.png)

*Fig. 2a* Gas Absorption Response of (a) Control System, (b) Sodium Carbonate Solution

![Figure 2b](image2.png)
**CO₂ Assimilation/Utilization Under Controlled Carbon Dioxide**

Figure 3 shows successful growth utilizing the three media under a controlled environment where the stationary phase was achieved; all systems also resulted in high values of $\mu_{\text{max}}$. Furthermore, as can be seen from Figure 4, bicarbonate concentration decreased, indicating its consumption, while carbonate concentration increased, signifying regeneration. It can be inferred that BICCAPS is taking place in the system.

**Table 3. Summary of Parameters of Control System Under BICCAPS**

<table>
<thead>
<tr>
<th></th>
<th>Biomass Productivity</th>
<th>$\mu_{\text{max}}$</th>
<th>Inorganic Carbon Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZC</td>
<td>10.42</td>
<td>0.0655</td>
<td>0.292</td>
</tr>
<tr>
<td>NC</td>
<td>10.51</td>
<td>0.0601</td>
<td>0.250</td>
</tr>
<tr>
<td>AC</td>
<td>10.81</td>
<td>0.0755</td>
<td>0.220</td>
</tr>
</tbody>
</table>

**Fig. 3** Growth of *Spirulina Platensis* in BICCAPS under Modified Zarrouk’s, NPK-based, and NPK-A₅ media

Dissolved carbon content, on the other hand, (Figure 5) decreases in concentration and increases thereafter. This is consistent with the provided carbonate equilibria where, at high pH values, carbon dioxide takes the form of bicarbonate. A summary of the biomass productivity, maximum specific growth rate, and inorganic carbon conversion values are tabulated in Table 3.

**Fig. 4** Concentration Profile of Carbonate and Bicarbonate in BICCAPS for controlled set-up under (a) Modified Zarrouk’s, (b) NPK-based, and (c) NPK-A₅ media
**CO₂ Assimilation/Utilization Under BICCAPS**

*S. Platensis* was successfully grown in all culture media, as seen in Figure 6 for ZS, NS, and AS systems.

Although growth was evident in all systems, only ZS and AS were able to fully utilize the bicarbonates, as seen in Figure 7a and 7c, wherein there is an observable decrease in bicarbonates and increase in carbonates. Hence, NS is not viable under the BICCAPS for *Spirulina platensis*. As for the dissolved carbon dioxide present in the systems, trends are presented in Figure 8. A summary of the different parameters for sodium carbonate is displayed in Table 4.

**Fig. 5** Dissolved Carbon Dioxide in BICCAPS for controlled set-up under (a) Modified Zarrouk’s, (b) NPK-based, and (c) NPK-A₅ media

**Fig. 6** Growth of *Spirulina Platensis* in BICCAPS with Na₂CO₃ under Modified Zarrouk’s, NPK-based, and NPK-A₅ media

**Fig. 7** Concentration profile of carbonate and bicarbonate in BICCAPS with Na₂CO₃ under (a) Modified Zarrouk’s, (b) NPK-based, and (c) NPK-A₅ media

**Comparison of Different Culture Media Under BICCAPS**

In an open system, NPK with A₅ flourished as compared to Modified Zarrouk’s and NPK-based, which have similar biomass productivities and *μ*ₘₐₓ. Under BICCAPS, modified Zarrouk’s media produced the highest productivity; therefore, proving that modified Zarrouk’s
remains to be the most suitable culture medium to be used while NPK-based medium produced relatively low productivities and maximum specific growth rates.

Fig. 8 Dissolved CO$_2$ in BICCAPS with Na$_2$CO$_3$ under (a) Modified Zarrouk’s, (b) NPK-based, and (c) NPK-A$_5$ media

Table 4. Summary of Parameters of Sodium Carbonate System Under BICCAPS

<table>
<thead>
<tr>
<th>Medium/ Carbon source</th>
<th>Biomass Productivity (mg L$^{-1}$ day$^{-1}$)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zarrouk’s medium/ CO$_2$- air</td>
<td>457.5</td>
<td>(Kumari et al., 2014)</td>
</tr>
<tr>
<td>NPK-10:26:26/ CO$_2$- air</td>
<td>422.5</td>
<td>(Delrue et al., 2017)</td>
</tr>
<tr>
<td>Modified Zarrouk’s medium/ HCO$_3$</td>
<td>55</td>
<td>(Madkour et al., 2012)</td>
</tr>
<tr>
<td>Modified Zarrouk’s medium/ HCO$_3$</td>
<td>0.052</td>
<td>(Madkour et al., 2012)</td>
</tr>
<tr>
<td>NPK-based with A$_5$/ CO$_2$-air</td>
<td>22.08</td>
<td>This study</td>
</tr>
<tr>
<td>NPK-based with A$_5$/ BICCAPS</td>
<td>6.80</td>
<td>This study</td>
</tr>
<tr>
<td>Modified Zarrouk’s medium/ BICCAPS</td>
<td>6.56</td>
<td>This study</td>
</tr>
</tbody>
</table>

In general, all control systems obtained high biomass productivities but lowest carbon conversion values. Relatively small carbon conversion is due to the low bicarbonate loading while biomass productivity increases due to the absence of inhibition caused by metal ions. *Spirulina platensis* under Zarrouk’s medium provided the highest carbon conversion among all three media used.

As for the carbonate systems, AS and ZS exhibited successful and similar biomass productivities and carbon conversion rates. Therefore, ZS and AS were deemed viable for *Spirulina platensis* utilization under BICCAPS. Moreover, the top system considering both parameters is ZS, making it the most suitable candidate for BICCAPS for the growth of *S. platensis*. NPK with A$_5$ solution, on the other hand, may be used as a sufficient cost-effective alternative to modified Zarrouk’s.

Upon comparing results with those present in literature, a large difference can be observed. Table 5 summarizes the different biomass productivities obtained from various studies.

Table 5. BICCAPS literatures for *S. platensis* at different conditions

<table>
<thead>
<tr>
<th>Medium/ Carbon source</th>
<th>Biomass Productivity (mg L$^{-1}$ day$^{-1}$)</th>
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<td>6.80</td>
<td>This study</td>
</tr>
<tr>
<td>Modified Zarrouk’s medium/ BICCAPS</td>
<td>6.56</td>
<td>This study</td>
</tr>
</tbody>
</table>

It can be noted from the table that studies involving CO$_2$ as carbon source produced superior results as compared to closed systems.

If studies involving Zarrouk’s medium are compared, it can be seen that the study conducted by Kumari et al. (2014) produced best results with intending to concoct the best mixture of CO$_2$-air and
It is followed by the study of Delrue et al. (2017) with the intention of large-scaling S. platensis production. Both studies surpass the results obtained from this study. However, it generated better results as compared to Madkour, Kamil, and Nasr's (2012) research, which aims to reduce the cost of Zarrouk’s medium by replacing all nutrients with more cost-effective alternatives. It is highly possible that modifications applied in the Zarrouk’s medium greatly affected the productivities.

Comparing studies under NPK fertilizers, once again, Kumari et al. (2014) produced the highest productivity, followed by this study’s enhanced NPK medium and NPK-based medium. In general, the cost-effective fertilizers depict as a promising alternative to the costly Zarrouk’s medium.

**CONCLUSIONS**

This study was able to identify that *Spirulina platensis* under BICCAPS was successful by portraying positive biomass productivities and carbon conversion values. In general, control systems resulted in the highest productivities and lowest carbon conversions. In a system of no carbonate salt, Modified Zarrouk’s medium produced the highest productivity. For carbonate systems, on the other hand, NPK with A₅ solution together with Modified Zarrouk’s medium displayed the highest biomass productivities as well as carbon conversions. It is therefore inferred that the growth of *S. platensis* under BICCAPS is best applied in a Modified Zarrouk’s medium, closely followed by NPK with A₅ solution as a cost-effective alternative.

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