Supplement of

Isotopic evidence for biogenic molecular hydrogen production in the Atlantic Ocean

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Due to the experimental upset and timing the following assumption have been made:

- The glass vessel is initially under vacuum.
- The water sample flows into the glass vessel, equilibrates with the headspace and mix completely with the injected makeup gas.
- No significant exchange between the headspace / makeup gas mixture and the water phase will happen until the sample has been extracted into an evacuated flask.

The following abbreviations and definitions have been used:

- $V_h$: volume headspace in the glass vessel (1.6 dm$^3$)
- $V_w$: volume water in the glass vessel (8.4 dm$^3$)
- $V_i$: volume gas transfer system (0.025 dm$^3$)
- $V_f$: volume flask and drying tube (1.1 dm$^3$)
- $p_h$: pressure headspace after transfer from Niskin bottle to glass vessel
- $p_{htm}$: pressure headspace and gas transfer system before addition of makeup gas
- $p_{htm}$: pressure headspace and gas transfer system after addition of makeup gas
- $p_c$: pressure headspace, gas transfer system and flask after expansion to flask
- $c_{w0}$: initial H$_2$ concentration in seawater
- $c_h$: H$_2$ concentration in headspace after transfer from Niskin bottle to glass vessel
- $c_w$: H$_2$ concentration in seawater after transfer from Niskin bottle to glass vessel
- $y_h$: H$_2$ wet mole fraction in headspace after transfer from Niskin bottle to glass vessel
- $y_{htm}$: H$_2$ dry mole fraction makeup gas
- $y_{htm}$: H$_2$ wet mole fraction in headspace and gas transfer system after addition of makeup gas
- $y_f$: H$_2$ dry mole fraction sample flask
- $R$: ideal gas constant (= 8.31446621 J K$^{-1}$ mol$^{-1}$)
- $T$: ambient absolute temperature (assumed to be equal to the gas temperature)

Defining the extraction efficiency $\eta$ as

$$\eta = \frac{c_h V_h}{c_{w0} V_w}$$  \hspace{1cm} (1)

the initial concentration of H$_2$ in seawater, $c_{w0}$, can be calculated from

$$c_{w0} = \frac{c_h V_h}{\eta V_w}$$  \hspace{1cm} (2)

The concentration in the headspace, $c_h$, was not measured directly, but can be derived from the measured H$_2$ mole fraction in the sampling flask. The sampling procedure following gas extraction under vacuum can be broken into three steps (see Methods section):

1. Expansion of headspace
2. Addition of makeup gas
3. Expansion of headspace/makeup gas mixture into sampling flask
**Step 1**: The volume of the headspace increases from $V_h$ to $V_h + V_t$, where $V_t$ is the volume of the gas transfer system. The total pressure drops from $p_h$ to $p_{htm}$ = $p_h$ $V_h$ / ($V_h + V_t$). H₂ mole fraction, $y_h$, and isotope delta, $\delta_h$, do not change.

**Step 2**: Adding makeup gas changes the total pressure from $p_{htm}$ to $p_{hnm}$. H₂ mole fraction and isotope delta also change. The H₂ mole fraction after mixing with makeup gas is:

$$y_{hnm} = (y_h - y_m) \frac{p_{htm}}{p_{hnm}} + y_m$$

(3)

The initial headspace H₂ concentration is calculated using the ideal gas law:

$$c_h = \frac{y_h p_h}{RT} = \frac{p_h}{RT} \left( y_{hnm} - y_m \right) \frac{p_{hnm}}{p_{htm}} + y_m$$

$$= \frac{1}{RT} \left( y_{hnm} - y_m \right) p_{hnm} \left( 1 + \frac{V_f}{V_h} \right) + y_m p_h$$

(4)

**Step 3**: The gas is expanded from the manifold into a sampling flask, passing through a drying trap. At this stage the mole fractions are changed due to drying of the gas. The volume of the system also changes, but this affects the H₂O and H₂ mole fractions by the same ratio.

Thus, $y_{hnm}$ is calculated from the measured dry mole fraction in the flask, $y_f$, as

$$y_{hnm} = y_f \left[ 1 - \frac{p_h (H_2O)}{p_{hnm}} \frac{V_h}{V_h + V_t} \right]$$

(5)

where $p_h (H_2O)$ is the initial water vapour pressure in the headspace. Due to the short time periods involved (< 4 min), water vapour equilibration is assumed not to occur upon subsequent pressure changes in the system.

Combining equations (2), (4) and (5), the initial seawater concentration can be calculated as

$$c_{w0} = \frac{(y_{hnm} - y_m)(V_h + V_t) p_{hnm} + y_m V_h p_h}{\eta V_w RT}$$

$$y_f \left[ \left( 1 + \frac{V}{V_h} \right) p_{hnm} - p_h (H_2O) \right] - y_m \left[ \left( 1 + \frac{V}{V_h} \right) p_{hnm} - p_h \right]$$

$$= \frac{\eta V_w RT}{y_f}$$

(6)

$y_h, y_m, p_{hnm}, V_t, V_h$ and $V_w$ were measured. $R$ is a constant (= 8.31446621 J K⁻¹ mol⁻¹).

$T$ is the ambient lab temperature and assumed to be equal to the gas and water temperature. Since ambient lab temperature and surface water temperature were never more than 1.2 °C apart, this assumption is unlikely to cause and error of more than 0.4 % in $c_{w0}$.

$p_h (H_2O)$ was assumed to be equal to the equilibrium vapour pressure (calculated following Green and Carritt (1967)) of seawater at temperature $T$ and at its actual salinity, which was measured to within 0.01 g kg⁻¹.

The extraction efficiency, $\eta$ can be calculated from the following mass balance

$$V_w c_{w0} = V_h c_h + \eta V_w c_h$$

(7)
Assuming that headspace and water phase are in equilibrium, the ratio of the H\textsubscript{2} concentration in water and in the headspace is given by the Ostwald coefficient

\[ \alpha = \frac{c_w}{c_h} \]  

(8)

This gives for the initial concentration in the water

\[ c_{w0} = \left( \frac{V_h}{V_w} + \alpha \right) c_h \]  

(9)

and for the extraction efficiency as defined in equation (2)

\[ \eta = \left( 1 + \alpha \frac{V_w}{V_h} \right)^{-1} \]  

(10)

In the present case, \( \alpha = a(\text{H}_2) \) was equal to 0.0163±0.0001, which gives \( \eta = 92 \% \) for \( V_w/V_h = 8.4/1.6 = 5.25 \).

\( p_h \) was not measured and had to be estimated from the gas concentrations in the water.

\[
p_h = p_h(N_2) + p_h(O_2) + p_h(Ar) + p_h(CO_2) + p_h(H_2O) \\
= RT \left[ c_h(N_2) + c_h(O_2) + c_h(Ar) + c_h(CO_2) \right] + p_h(H_2O) \\
= RT \left[ \frac{c_{w0}(N_2)}{V_h} + \frac{c_{w0}(O_2)}{V_h} + \frac{c_{w0}(Ar)}{V_h} + \frac{c_{w0}(CO_2)}{\alpha(CO_2)} \right] + p_h(H_2O) 
\]

(11)

The extraction efficiencies of all gases were assumed to follow their solubilities. Due to the buffering effect of the CO\textsubscript{2} system (CO\textsubscript{2}(w), H\textsubscript{2}CO\textsubscript{3}, HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{3}\textsuperscript{2-}), the dissolved CO\textsubscript{2} concentration changes by only 1 or 2 \% during extraction, so that the headspace concentration can be estimated from \( c_{w0}(CO_2)/\alpha(CO_2) \).

\( p_h \) was estimated to vary between 98 and 111 hPa, with contributions of 46 to 52 hPa from N\textsubscript{2}, 23 to 27 hPa from O\textsubscript{2}, 1.1 to 1.3 hPa from Ar, 0.4 hPa from CO\textsubscript{2} and 17 to 40 hPa from H\textsubscript{2}O.

Based on equation (4), the isotope delta of the headspace gas can be calculated as follows

\[
\delta_h = \frac{\delta_{hnm} V_{hnm} \left( 1 + \frac{V_h}{V_h} \right) p_{hnm} - \delta_{mnm} V_{mnm} \left( 1 + \frac{V_h}{V_h} \right) p_{hnm} - p_h}{\left( 1 + \frac{V_h}{V_h} \right) p_{hnm} - p_h} \]  

(12)

Two alternative scenarios were considered to derive the initial isotope delta of dissolved H\textsubscript{2}, with scenario 1 assuming equilibrium isotopic fractionation between headspace and water, and scenario 2 assuming kinetic isotopic fractionation during extraction from Niskin bottle to glass vessel.

**Scenario 1:** Correction of \( \delta_h \) for equilibrium isotopic fractionation between headspace and water
The equilibrium isotope fractionation between water and headspace is defined as

$$\varepsilon = \frac{1 + \delta_w}{1 + \delta_h} - 1$$  \hspace{1cm} (13)

The isotopic mass balance equation between headspace and water can then be described via

$$V_w C_w \delta_{w0} = V_h C_h \delta_h + V_w C_w \delta_w$$  \hspace{1cm} (14)

which gives for the isotope delta of the original dissolved gas:

$$\delta_{w0} = \frac{\varepsilon (1 + \delta_h)}{1 + \frac{V_h}{V_w} \alpha} + \delta_h$$  \hspace{1cm} (15)

The equilibrium isotope fractionation between dissolved phase and gas phase is $\varepsilon = (37 \pm 1) \text{‰}$ at 20 ºC (Knox et al., 1992). The temperature dependence of $\varepsilon$ is unknown and was neglected here.

**Scenario 2:** Correction for kinetic isotopic fractionation between headspace and water

If kinetic isotope fractionation applies, then the isotopic composition of the residual gas in the water is

$$\delta_w = (1 + \delta_{w0}) \left( \frac{C_w}{C_{w0}} \right)^{\varepsilon_k} - 1 = (1 + \delta_{w0}) \left( 1 - \eta \right)^{\varepsilon_k} - 1$$  \hspace{1cm} (16)

Then, the isotope delta of the accumulated gas in the headspace is

$$\delta_h = (1 + \delta_{w0}) \frac{1 - (1 - \eta)^{1_{\varepsilon_k}}}{\eta} - 1$$  \hspace{1cm} (17)

which gives for the isotope delta of the original dissolved gas:

$$\delta_{w0} = \frac{(1 + \delta_h) \eta}{1 - (1 - \eta)^{1_{\varepsilon_k}}} - 1$$  \hspace{1cm} (18)

The kinetic isotope fractionation during gas evasion is $\varepsilon_k = (-18 \pm 2)\text{‰}$ at 20 ºC (Knox et al., 1992). The temperature dependence of $\varepsilon_k$ is unknown and was neglected here.

**References:**

