Supplement of

Isotopic evidence for biogenic molecular hydrogen production in the Atlantic Ocean

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Supplement

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\(^3\))
• \( V_t \): volume gas transfer system (0.025 dm\(^3\))
• \( p_h \): pressure headspace after transfer from Niskin bottle to glass vessel
• \( p_{ht} \): pressure headspace and gas transfer system before addition of makeup gas
• \( p_{htm} \): pressure headspace and gas transfer system after addition of makeup gas
• \( p_f \): pressure headspace, gas transfer system and flask after expansion to flask
• \( c_{w0} \): initial \( \text{H}_2 \) concentration in seawater
• \( c_h \): \( \text{H}_2 \) concentration in headspace after transfer from Niskin bottle to glass vessel
• \( c_w \): \( \text{H}_2 \) concentration in seawater after transfer from Niskin bottle to glass vessel
• \( y_h \): \( \text{H}_2 \) wet mole fraction in headspace after transfer from Niskin bottle to glass vessel
• \( y_{tm} \): \( \text{H}_2 \) dry mole fraction makeup gas
• \( y_{htm} \): \( \text{H}_2 \) wet mole fraction in headspace and gas transfer system after addition of makeup gas
• \( y_f \): \( \text{H}_2 \) 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

\[
\eta = \frac{c_h V_h}{c_{w0} V_w}
\]  

(1)

the initial concentration of \( \text{H}_2 \) in seawater, \( c_{w0} \), can be calculated from

\[
c_{w0} = \frac{c_h V_h}{\eta V_w}
\]  

(2)

The concentration in the headspace, \( c_h \), was not measured directly, but can be derived from the measured \( \text{H}_2 \) 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 \( p_{ht} = p_h \frac{V_h}{V_h + V_t} \). H\(_2\) mole fraction, \( y_h \), and isotope delta, \( \delta_h \), do not change.

Step 2: Adding makeup gas changes the total pressure from \( p_{ht} \) to \( p_{htm} \). H\(_2\) mole fraction and isotope delta also change. The H\(_2\) mole fraction after mixing with makeup gas is:

\[
y_{htm} = (y_h - y_m) \frac{p_{ht}}{p_{htm}} + y_m
\]

The initial headspace H\(_2\) concentration is calculated using the ideal gas law:

\[
c_h = \frac{y_h p_h}{RT} = \frac{p_h}{RT} \left[ (y_{htm} - y_m) \frac{p_{htm}}{p_{ht}} + y_m \right]
\]

\[
= \frac{1}{RT} \left[ (y_{htm} - y_m) p_{htm} \left(1 + \frac{V_h}{V_t}\right) + y_m p_h \right]
\]

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 H\(_2\)O and H\(_2\) mole fractions by the same ratio.

Thus, \( y_{htm} \) is calculated from the measured dry mole fraction in the flask, \( y_f \), as

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

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

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

\[
y_f \left[ \left(1 + \frac{V_f}{V_h}\right) p_{htm} - p_h(H_2O) \right] - y_m \left[ \left(1 + \frac{V_t}{V_h}\right) p_{htm} - p_h \right]
\]

\[
= \frac{1}{\eta V_w RT}
\]

\( y_h, y_m, p_{htm}, V_t, V_h \) and \( V_w \) were measured. \( R \) is a constant (= 8.314446621 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 & Carritt (1967 #1602)) 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

\[
V_w c_{w0} = V_h c_h + cV_w c_h
\]
Assuming that headspace and water phase are in equilibrium, the ratio of the \( H_2 \) 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, \( \alpha = \alpha(H_2) \) 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.

\[
p_h = p_h(N_2) + p_h(O_2) + p_h(Ar) + p_h(CO_2) + p_h(H_2O)
\]

\[
= RT \left[ c_h(N_2) + c_h(O_2) + c_h(Ar) + c_h(CO_2) \right] + p_h(H_2O)
\]

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

(11)

The extraction efficiencies of all gases were assumed to follow their solubilities. Due to the buffering effect of the \( CO_2 \) system (\( CO_2(w), H_2CO_3, HCO_3^- \) and \( CO_3^{2-} \)), the dissolved \( CO_2 \) 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 \( N_2 \), 23 to 27 hPa from \( O_2 \), 1.1 to 1.3 hPa from \( Ar \), 0.4 hPa from \( CO_2 \) and 17 to 40 hPa from \( H_2O \).

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

\[
\delta_h = \frac{\delta_{h\text{m}} y_{h\text{m}} \left( 1 + \frac{V_h}{V_w} \right) p_{h\text{m}} - \delta_{m\text{v}} y_m \left( 1 + \frac{V_h}{V_w} \right) p_{h\text{m}} - p_h}{y_{h\text{m}} \left( 1 + \frac{V_h}{V_w} \right) p_{h\text{m}} - y_m \left( 1 + \frac{V_h}{V_w} \right) p_{h\text{m}} - p_h}
\]  

(12)

Two alternative scenarios were considered to derive the initial isotope delta of dissolved \( H_2 \), 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

\[ \varepsilon = \frac{1 + \delta_w}{1 + \delta_h} - 1 \]  

(13)

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

\[ V_w c_{w0} \delta_{w0} = V_h c_h \delta_h + V_w c_w \delta_w \]  

(14)

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

\[ \delta_{w0} = \frac{\varepsilon(1 + \delta_h) + \delta_h}{1 + \frac{V_h}{V_w} \alpha} \]  

(15)

The equilibrium isotope fractionation between dissolved phase and gas phase is \( \varepsilon = (37 \pm 1) \) ‰ 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

\[ \delta_w = (1 + \delta_{w0}) \left( \frac{c_w}{c_{w0}} \right)^{\varepsilon_k} - 1 = (1 + \delta_{w0}) \left( 1 - \eta \right)^{\varepsilon_k} - 1 \]  

(16)

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

\[ \delta_h = (1 + \delta_{w0}) \frac{1 - (1 - \eta)^{\varepsilon_k}}{\eta} - 1 \]  

(17)

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

\[ \delta_{w0} = \frac{(1 + \delta_h) \eta}{1 - (1 - \eta)^{\varepsilon_k}} - 1 \]  

(18)

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