General Comments

Pantoja et al examined the hydrolytic potential for carbon molecules in the water column and sediments near the Itata River in central Chile. This was attempted through measurements of leucine, glucose, and cellobiose hydrolysis rates (activity). The 5 years of activity data are combined and integrated through the water column, but separated into winter and spring+summer periods. Nitrate, chl, temperature, and salinity are separated spatially and seasonally and used to highlight the unique upwelling regime found in the region.

In my opinion, this paper serves three central purposes:
1) it provides hydrolysis rates for the water column and sediment surface in the Itata River and upwelling area
2) it highlights the seasonal differences in activity between winter and summer and along the shelf
3) it predicts the presence of excess hydrolysis potential compared to primary production

The authors do a respectable job of presenting the available data necessary to discuss the above points. As is often the case, more data is required for an in-depth analysis of some of the hypotheses. Unfortunately, the data required to address some of the primary questions involved with the study were lacking, especially regarding the excess hydrolysis potential. In addition, I have strong reservations as to the validity of the hydrolysis potential (carbon budget)model; these points are outlined in the Specific Comments section below. It is, however, an interesting and intriguing method to quantify organic carbon transport.

Their work can be of use to others attempting a closer examination of the biogeochemistry of the Itata River or Chilean upwelling systems. This manuscript does not analyze the regulation or expression of hydrolysis activity in the area, but rather provides a spatially explicit, quantitative description of activity. However, there is limited discussion as to what relationship activity may have to other biogeochemical parameters (oxygen, chlorophyll, etc).

While the paper is perfectly understandable, there are numerous errors in grammar and consistency in capitalization (listed in Technical Comments below). That said, the required changes should be quick and easy to make.

There are major points within this paper that I would like to see addressed before publishing regarding the methodology and interpretation of some of the data. However, I think the work Pantoja et al presented here can be of use to the aquatic science community and fits within the theme of Biogeosciences.

Specific Comments

* I am wondering if there was a specific reason MUF-glu and MUF-cel were used instead of a non-glucose substrate (like a lipid) to extend the idea of carbon degradation further. Are there any known

differences in the hydrolytic activity between single glucose and multiple glucose substrates? This paper almost always lumped the two together and the data is very similar between the two.

*P1342 L4-11: I would like to see a more detailed description of the enzyme methods. I am assuming the enzyme activity measurements were made immediately after taking the water samples (if that isn't the case, the conditions of the treatment need to be specified). Was anything done to keep the samples at in situ temperature during the incubation? The 5 mL of water used in the assay will almost certainly be a higher temperature after a 6hr incubation on board, which would likely substantially increase the activity. The fluorescence of the standards (and fluorescence in general) is probably also temperature dependent, so a different calibration curve would be needed for each new batch of standard (or refrigeration prior to measurement to adjust everything to a standard temperature). Was anything done to the seawater the standards were made out of, like filtering out the biomass to prevent attenuation? What were the filter settings? Was the water for the standards taken fresh for every sample? Was a new batch of substrate made for each measurement? Dissolved in distilled water or filtered seawater?

*There is no mention of regressing enzyme activity against primary production or chl-a concentration and verifying if there is a relationship and/or if the relationship has seasonal variations. It would increase the strength of your claim for primary productivity driving enzyme activity if such a relationship could be shown or at least discussed. It's also relevant to the carbon budget model, since enzyme activity would then be expected to change in tandem with the phytoplankton population

*p1342 L10-19:

1) Especially for short incubations, the increase in product is usually close to linear; is there sufficient evidence from your incubations that a first-order model is better? Michaelis-Menton kinetics, which most hydrolytic enzymes seem to follow, are equivalent to the first-order model under conditions when [S]<Km, so I can see where that might come from, though it doesn't appear any kinetics were run for this region.

For demonstration purposes, using the first-order model and the high peptidase hydrolysis rates (and back-calculating k from the activity and substrate concentration):
C=10uM*e^(-0.0182 h^{-1}*6h)=8.97 uM
That's a difference of: 10 – 8.97 = 1.03 uM substrate used up over 6 hrs. If we plug the two concentrations into the model at the beginning of the time series, where the difference in substrate concentration has the largest effect:
V(10 uM) = k[C0] = 0.0182 h^{-1} * 10uM = 182 nM h^{-1}
V(8.97 uM) = 0.0182 h^{-1} * 8.97 uM = 163 nM h^{-1}

If one uses a linear model with the rate given above (182 nM hr^{-1}), then over 6 hrs we expect to see 1.09 uM produced, which is 60 nM more than the decay model predicts and an addition 10 nM per hour (average). That's not a large difference; it would be nice to see an example of the data showing the curvature, since that's definitely different than the way most people calculate enzyme activity.

2) The rate constants are stated as being based on the first order equation: $k = \ln(C_0/C_0\text{-}P)/t$, which is equivalent to $C_0=C*e^{kt}$. As written, this has the product exponentially increasing with time, which is probably not correct. From this I am assuming the original equation has C_0 and C reversed from their usual positions in a first-order decay equation, and a missing negative sign on the kt term. Since the experiments measure the increase in fluorescent products, the equation would better reflect the increase in product and not the decrease in substrate concentration: $P = C_0(1\text{-}e^{-kt})$.

*Figure 4, panel A, the datum furthest offshore (far right): There appears to be only 1 data point, but the average is much higher than the data point. In addition, the standard deviation range is much larger than the variability of the blue dot(s) for that point. Both suggest there is some missing data for this station. Also, I would rescale the bottom 4 panels; there's no data above 61 nM h^{-1}, so no reason to keep all the data too close together. A short note in the caption could alert readers of the scale change.

*Figure 6: The paper mentions a lack of a trend after the outflow was introduced in enzyme activity. While there isn't much data to really test such a hypothesis, I agree there doesn't seem to be much going on with surface water. However, all of the data points in the surface sediment are higher than all but one data point after the outflow is installed; could that be significant? The outflow is below the surface, at 30 m, which is also below the spring-summer thermocline. Many of the low surface values occur in the spring-summer when the thermocline is in place. Perhaps bacteria or substrate is trapped below preventing a surface signal but still present and able to create the sediment signal?

*p1349 L22: The substrate concentration was 10 uM for MCA-Leu. However, the measured protein concentration was significantly less at 1-2 uM (and well below enzyme saturation). In both Michaelis-Menten kinetics and the first-order model used here, the initial hydrolysis rates are sensitive to substrate concentration. If we assume the substrate concentration is equal to the measured surface proteins (no free amino acids and all measured amino acids are the only valid substrates), but keeping the same k values (~0.0182 for MCA-Leu), the hydrolysis rate drops from go from 182 nM h^{-1} (10uM substrate) to 36.4 nM h^{-1}, or an 80% drop. An 80% decrease in the integrated hydrolysis activity in the model puts primary production well ahead of hydrolysis substrate, opposite of the main argument of this section. Even ignoring in situ substrate concentrations, the error bars of primary production for both seasons are sufficient to equal the hydrolysis rates; I don't see how the numbers given in Fig 7 can be interpreted to say they definitively show a substrate deficit.

* Contrary to the induction model of these enzymes (expressed in order to scavenge nutrients), your data shows activity related to high substrate concentration. If the river is a major substrate transport pathway, then activity should be higher in regions of freshwater. Is this the case? For instance, if river flow is high in winter (more nutrients) but there is low biomass (no sun), then what does activity do? You would have to restrict the enzyme data to the surface waters within 6km of the shore instead of integrating through the water column like the reported data, but that should give a decent idea on how river flow may directly influence enzyme activity.

Technical Corrections

*p1336 L1: “The response…was evaluated…” I’m not sure “response” is the correct term here. Perhaps just rephrase it to say you were evaluating the degradation capability of macromolecules off central Chile, an environment influenced by both river discharge and upwelling, by estimating rates of microbial enzymatic hydrolysis.

-L7: “…activity with the substrate MCA-leu…”

- L7-8: either hyphenate all number ranges or use “to” for each range

- L13: I think it is better to say that MCA-leu had the same range of values as MUF-leu, since MUF-cel alone ranged over 3 orders of magnitude.

- L20: “…with high organic molecule availability…”

*p1338 L2 “…Sanchez et al. (2008) observed…” Since not all of the authors in the reviewed paper are authors in Sanchez et al, using “we” isn’t really correct.

*p1339 L9 “We aim to obtain…”

*p1340 L10: “195 km”

*-L13 “…a major pulp mill was installed.”

-L19: “Twenty-nine locations were visited:…”

- L20-23: “Surface seawater and river samples were collected with Niskin bottles and polypropylene carboys, which were used for nutrient, chlorophyll-*a*, and protein measurements. Surface water was additionally used for estimating the rates of primary production and extracellular hydrolysis of proteins and carbohydrates.” Or something similar to that.

-L25: “Sediment samples were collected with a box corer or a Van Veen grab, the surface (0-1 and 1-2 cm) sediment removed, and stored…” This edit specifies that more than just the top sediment was taken by the coring devices.

*p1341 L7: “Parsons et al., 1984” The paper currently has an ‘i’ instead of a one.

-L8-9: “..proteins by high performance liquid chromatography (HPLC). Particulate proteins were analyzed as Total Hydrolyzable Amino Acids by a Shimadzu HPLC coupled to an on-line fluorescence detector with 330 nm (excitation) and 450 nm (emission) filters and an autosampler.” The use of ‘HPLC’ and ‘fluorescence detector’ was redundant.

*p1342 L6-7: You use both lowercase and capital abbreviations for substrates, i.e. MCA-leu and MCA-Leu.

*p1343 L8-10: “…during winter, cold water temperatures… In contrast, both spring-summer temperatures (>13°C) and a stratified water column were observed…”

-L10: “The horizontal distribution…”

-L13: “In contrast, winter temperatures remained…”

*p1345 L9: a salinity gradient, or transect/line/section

*p1347 L22 and others: aminopeptidase (all one word)
-L27-29: I don't understand the part about the limitation in microbial activity and how that changes the interpretation of the protein and sugar degradation data. Can you change the wording?

*p1348 L24: "since the lack of a trend"

*p1349 L21: "allocthonous"

*Results section: make sure the units for concentration (oxygen, nitrate, proteins) are uM and not um.

*Table 2: put data in sequential order by date (i.e. August 2006 goes before April 2008). Or, group by winter vs spring/summer. Also specify the years of the other data. For instance, what year is the first January measurement from?*Fig 4,5 legend: MCA-glu (A,B) should be MCA-leu (or MCA-Leu). Also, enzyme rates were previously reported as nM h-1, not nmol L-1 h-1.

*Fig 1: Add note in legend that paper mill outflow is located near Station 6.