Anaerobic biodegradability of fish remains: experimental investigation and parameter estimation

Andres Donoso-Bravo, Francoise Bindels, Patrick A. Gerin and Alain Vande Wouwer

ABSTRACT

The generation of organic waste associated with aquaculture fish processing has increased significantly in recent decades. The objective of this study is to evaluate the anaerobic biodegradability of several fish processing fractions, as well as water treatment sludge, for tilapia and sturgeon species cultured in recirculated aquaculture systems. After substrate characterization, the ultimate biodegradability and the hydrolytic rate were estimated by fitting a first-order kinetic model with the biogas production profiles. In general, the first-order model was able to reproduce the biogas profiles properly with a high correlation coefficient. In the case of tilapia, the skin/fin, viscera, head and flesh presented a high level of biodegradability, above 310 $mLCH_4$ $gCOD^{-1}$, whereas the head and bones showed a low hydrolytic rate. For sturgeon, the results for all fractions were quite similar in terms of both parameters, although viscera presented the lowest values. Both the substrate characterization and the kinetic analysis of the anaerobic degradation may be used as design criteria for implementing anaerobic digestion in a recirculating aquaculture system.

Key words | anaerobic digestion, aquaculture, mathematical modeling, parameter estimation

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INTRODUCTION

Aquaculture has expanded almost twelve-fold worldwide in the last three decades, at an average annual rate of 8.8%. In 2010, global production of farmed fish was 59.9 million tons, up by 7.5% from 55.7 million tons in 2009 (FAO 2012). Recirculation aquaculture systems (RAS) have gained importance due to the comparative advantages over conventional flow through systems, especially in terms of the control that can be exerted over rearing conditions and the technical possibility of reducing water consumption and waste release by a factor of 100. The aquaculture production of sturgeon has risen significantly in the last decade, mainly as a way to offset the decline of natural stocks (Bronzi *et al.* 2011). On the other hand, tilapia and carp species have been introduced throughout the tropics and account for about 80% of tropical inland aquaculture production (Arthur *et al.* 2010).

An often overlooked issue is solid waste management in RAS in terms of processing residues from fish slaughter. The organic waste that originates from fish slaughter and cutting may account for as much as 50% of the total produced fish (Arvanitoyannis & Kassaveti 2008). Anaerobic digestion (AD) enables the treatment of biowaste and wastewater, the recovery of energy through biogas and the valorization of the remaining biosolids as organic fertilizer. It is considered to be a consolidated and sustainable technology in Europe (Baere *et al.* 2012). The application of AD in the aquaculture sector is still incipient, especially for substrates such as fish slaughter and carving wastes (Mirzoyan *et al.* 2010). This is due to the complex composition of the substrate, the significant protein fraction (55–75% of the dry matter (DM)) of the fish waste, which leads to low chemical oxygen demand (COD)/N ratios and ammonia inhibition. Therefore, most of the studies found in the literature use co-digestion of fish waste with another substrate as the way to overcome this issue (Bouallagui *et al.* 2009; Alvarez *et al.* 2010; Eiroa *et al.* 2012; Regueiro *et al.* 2012; Serrano *et al.* 2013). However, to the best of our knowledge, very few studies have evaluated the anaerobic biodegradability of fish waste as the sole carbon and nutrient source (Nges *et al.* 2012). This may become relevant when no other

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substrates are available or merely when co-digestion is not a feasible option for companies.

The biochemical methane potential (BMP) test represents the conventional procedure to evaluate the anaerobic biodegradability of any substrate fed into anaerobic digesters. This method is considered simple and reliable, and it allows the estimation of some important kinetic parameters (Donoso-Bravo *et al.* 2010). In AD modeling, when the hydrolysis step of the particulate organic matter is the rate-limiting step, a first-order equation may be used to estimate the hydrolysis rate and the biodegradability extent (Batstone *et al.* 2009). The hydrolysis rate is the average hydrolysis reaction rate, while the biodegradability extent assesses the biodegradable fraction of the substrate, i.e., the fraction of the substrate that will eventually be anaerobically biodegraded.

In this study, characterization of the composition and of the AD kinetics of the fish remains (FR) from the RAS of two popular market fish, tilapia and sturgeon, is evaluated. The various fractions that can be separated when processing fish carcasses were tested individually in order to get the background data for further modeling of various scenarios of waste treatment. The present paper focuses on using a simplified mathematical model to estimate the hydrolytic parameters related to the anaerobic degradation of each fraction.

MATERIAL AND METHODS

Substrate and inoculums

Tilapia and sturgeon FR were provided by the RAS aquaculture company, Belgian Quality Fish (Dottignies, Mouscron, Belgium). Both types of fish were processed manually using a cutting machine. In the case of tilapia, the obtained fractions were: (a) fishbone, (b) flesh, (c) scales, (d) skin, (e) head, and (f) viscera. For the sturgeon, they were: (a) fishbone, (b) flesh, (c) fin, (d) skin from the lower part of the body (belly), (e) skin from the upper part of the body (back), (f) head, and (g) viscera. Activated sludge from Chastre urban wastewater treatment plant, Mont-Saint-Guibert, Belgium, with a concentration of 13.7 gVS/L, was used to feed the anaerobic inoculum maintained in the laboratory. Before the test, the inoculum was incubated for 10 days in a 20 L reactor in order to acclimatize the microbial population. The content of total solids, volatile solids (VS), COD and pH were analyzed according to *Standard Methods* (APHA 1998). Total Kjeldahl nitrogen (TKN) was analyzed using the Kjeldahl method.

Anaerobic digestibility assays

Batch anaerobic digestibility assays were run in triplicate in 1 l Schott glass bottles with a total mixed liquor volume of 445 mL. The mixed liquor contained 7.5 gCOD_inoculum and 5 gCOD_inoculum/gCOD_substrate to ensure good pH buffer capacity and degradation activity. A blank test, containing only the inoculum and water to replace the substrate, were also run in order to subtract the biogas produced from the organic matter remaining in the inoculum. The anaerobic inoculum was produced by incubating activated sludge from the nearby urban wastewater treatment plant, as a substrate, with an anaerobic inoculum maintained in the laboratory. All experiments were carried out on an orbital shaker at 120 rpm, 2.5 cm amplitude at 35 °C in the dark. The biogas production was measured with a manometer (type UNIK 5000 PTX5072-TA-A3-CA-H0-PA, General Electric, distributed by Dimed, Antwerpen, Belgium). When the pressure was around or over 100 hPa, the biogas was collected and analyzed by gas chromatography, and the pressure released to the atmospheric pressure. The normalized volume of gas produced between time $t-1$ and time t was calculated for each gas (CH_4 or CO_2) using Equation (1):

$$V_{gas} = \frac{(P_t \cdot C_{gas,t} - P_{t-1} \cdot C_{gas,t-i}) \cdot V_{hs} \cdot 273.15}{1,013.25 \cdot 308.15} \quad (1)$$

where P_{t-1}, P_t (mbar) are the pressure in the headspace at time $t-1$ and t, respectively; $C_{gas\ t-1,}$ $C_{gas,\ t}$ are the molar (volume) fraction of gas (CO_2, CH_4) in the biogas at time $t-1$ and t, respectively; V_{hs} is the headspace volume [L]; 273.15 (K) and 1,013.25 (mbar) are the normal conditions temperature and pressure (0 °C, 1 atm); and 308.15 (K) is the incubation temperature (35 °C).

Kinetic parameters estimation

The gas production accumulated with time was modeled by first-order kinetics, according to Equation (2). This expression is commonly used in the case of anaerobic degradation of solid waste (as waste-activated sludge), where the hydrolysis reaction becomes the limiting reaction that governs the overall process:

$$B = B_0 \cdot (1 - \exp(-k_h \cdot t)) \quad (2)$$

where B is the cumulative gas production, t is the cumulative time from the start of the experiment, k_h is the hydrolysis constant, B_0 is the ultimate degradation extent (parameter related to the degradability of the substrate). For parameter estimation, experimental data from BMP tests are used and a direct-search procedure based on the Nelder–Mead algorithm (*Fminsearchbnd* toolbox coded in Matlab®) is used to explore the parameter space. In this case, a simple least squares criterion (Equation (3)) was chosen as the optimization criterion:

$$J(\theta) = \sum_{t=1}^{N} (y_{\text{exp}}(t) - y_{\text{sim}}(t, \theta))^2 \tag{3}$$

where J is the cost function, y_{exp} are the collected measurements, y_{sim} are the model-predicted outputs, θ represents the parameters to be determined, and N is the number of measurements. Once the cost function has been minimized with respect to the parameters θ, the accuracy of the estimated parameters $\hat{\theta}$ can be assessed using the inverse of the Fisher information matrix (Equation (4)), which gives a lower bound on the achievable parameter error covariance matrix (C_N):

$$C_N \succ (F(\theta))^{-1} \quad F(\theta) = \frac{1}{\hat{\sigma}^2} \sum_{i=1}^{N} \left[\frac{\partial y_i(t, \theta)}{\partial \theta}\right]^T \left[\frac{\partial y_i(t, \theta)}{\partial \theta}\right] \tag{4a}$$

In this expression, $\hat{\sigma}^2$ represents an estimate of the variance of the experimental data, which can either be known from an evaluation of the experimental procedure, or estimated *a posteriori* by:

$$\hat{\sigma}^2 = \frac{J(\hat{\theta})}{N-2} \tag{4b}$$

Finally, once the covariance matrix is available, an approximation of the standard deviation of the parameters can be obtained through Equation (5):

$$\sigma(\theta_i) = \sqrt{C_N(i, i)} \tag{5}$$

RESULTS AND DISCUSSION

Characteristics of fish fractions

For both tilapia and sturgeon, all fish fractions were manually separated and characterized individually. Table 1 summarizes the measured data.

The most striking difference between tilapia and sturgeon is the bone fraction composition, with a much higher DM and mineral content for tilapia than for sturgeon. While both species are bony fish, sturgeon bones are known to be much less calcified and almost cartilaginous (Maccari *et al.* 2010). For both fish, the head presents a higher mineral content, as compared to the other fractions. This can be attributed to the contribution of the head bones. By considering that proteins are usually defined as 6.25*TKN, (i.e. N represents 16% of 'average' proteins), the nitrogen contents presented in Table 1 indicate that most fractions have a high protein content (60–300 $g_{\text{protein}}/kg_{\text{WM}}$), especially the skin (mostly collagen). The very low mineral content (or the high VS content) and high nitrogen content of tilapia skin can be attributed to the loss of scales during fish pre-processing and the relatively high nitrogen content of collagen, as compared to the 'average' proteins (Hoemann Sun *et al.* 2002), while sturgeon has no significantly mineralized scales that can be lost during processing. The high COD/VS ratio observed for sturgeon viscera and flesh can be attributed to their high lipid contents.

Anaerobic degradation and parameter estimation of methane production

The biogas production rate of each fish fraction was monitored. Figures 1 and 2 present the cumulative methane production, together with the first-order-model fit Equation (2). The methane content of the biogas was in the range of 60–70% v/v for both fish and all fractions, with no clear relationship with the type of fraction. The results demonstrate that the anaerobic degradation of different fish fractions, as a sole carbon source, is feasible without the presence of a co-substrate in batch conditions. This outcome indicates that the transformation of fish solid waste into methane may represent a solution for reducing the energy cost, solid waste treatment and disposal for aquaculture factories. Nonetheless, it has to be borne in mind that the highest N concentration brought to the mixed liquor by the fish substrate was 0.3 gN/LML (for tilapia skin + fin), i.e., five times lower than the expected inhibition range. Therefore, dilution of the substrate plays an important role in the success of the digestion.

For all fractions and sludge of both fish, the first-order model is in good agreement with the experimental data, in most cases being inside the confidence interval of the data. This means that methane production for these substrates in batch tests can be reproduced by the model. The regression coefficient is above 0.97.

Table 1 | Characteristics of the fraction of sturgeon and tilapia

	Skin + Fin	Viscera	Bones	Head	Flesh	Scales	Fish residue[a]	
Tilapia								
Fish fraction (gWM/100 gWM carcass)	7.9	12.4	7.5	42.0	25.4	4.7	100	
DM (gDM/100 gWM)	34.2 ± 0.07	40.3 ± 0.76	58.0 ± 0.10	38.7 ± 0.03	21.1 ± 0.01	25.7 ± 0.00	34.9 ± 0.01	
VS (gVS/100 gDM)	89.3 ± 1.78	98.3 ± 0.08	40.7 ± 1.53	78.8 ± 0.65	94.0 ± 0.24	54.4 ± 0.04	81.8 ± 0.33	
COD (gCOD/gVS)	1.93 ± 0.06	2.46 ± 0.06	1.47 ± 0.06	1.99 ± 0.08	1.55 ± 0.01	1.29 ± 0.00	1.86 ± 0.04	
COD (gCOD/gWM)	0.59 ± 0.02	0.98 ± 0.02	0.35 ± 0.01	0.61 ± 0.02	0.31 ± 0.00	0.18 ± 0.00	0.53 ± 0.01	
N Kjeldahl (gN/kgWM)	47.0 ± 4.83	10.1 ± 0.70	36.7 ± 0.23	23.2 ± 1.35	30.6 ± 0.19	54.1 ± 3.1	27.8 ± 0.71	
Methane potential (mLCH$_4$/gCOD)	315 ± 3.89	317 ± 8.78	247 ± 16.56	321 ± 13.96	317 ± 10.31	202 ± 3.06	308 ± 6.64	
	Skin bottom	**Skin back**	**Viscera**	**Bones**	**Head**	**Flesh**	**Fin**	**Fish residue[a]?**
Sturgeon								
Fish fraction (gWM/100 gWM carcass)	7.2	11.6	16.6	6.8	32.5	14.1	11.0	100.0
DM (gDM/100 gWM)	35.5 ± 0.48	32.4 ± 0.52	32.4 ± 0.19	23.2 ± 3.5	29.6 ± 0.03	29.5 ± 0.09	27.3 ± 2.1	30.1 ± 0.48
VS (gVS/1000 gDM)	93.3 ± 0.23	93.0 ± 0.3	96.0 ± 0.04	87.3 ± 0.12	83.8 ± 0.20	96.2 ± 0.23	71.7 ± 0.57	88.6 ± 0.23
COD (gCOD/gVS)	1.79 ± 0.02	1.58 ± 0.013	2.07 ± 0.015	1.46 ± 0.045	1.68 ± 0.046	1.93 ± 0.083	1.44 ± 0.129	1.76 ± 0.047
COD (gCOD/gWM)	0.59 ± 0.010	0.48 ± 0.008	0.65 ± 0.053	0.30 ± 0.011	0.42 ± 0.024	0.55 ± 0.045	0.28 ± 0.019	0.47 ± 0.007
N Kjeldahl (gN/100 gDM)	9.0 ± 0.46	10.7 ± 1.06	6.6 ± 0.04	8.2 ± 0.14	6.5 ± 0.72	7.1 ± 0.17	8.7 ± 0.07	7.7 ± 0.77
N Kjeldahl (gN/kgWM)	32.08 ± 2.06	34.59 ± 3.91	21.52 ± 0.19	19.14 ± 3.23	19.40 ± 2.11	20.79 ± 0.55	23.69 ± 1.95	23.09 ± 1.84
Methane potential (mLCH$_4$/gCOD)	295 ± 2.9	306 ± 5.5	278 ± 1.7	303 ± 2.7	317 ± 7.04	301 ± 3.4	257 ± 3.9	297 ± 28.0

Mean ± deviation from the mean, duplicates (DM: dry matter, WM: wet crude matter, COD: chemical oxygen demand).
[a]Fish residue composition calculated by combining the proportional contribution of each fraction.

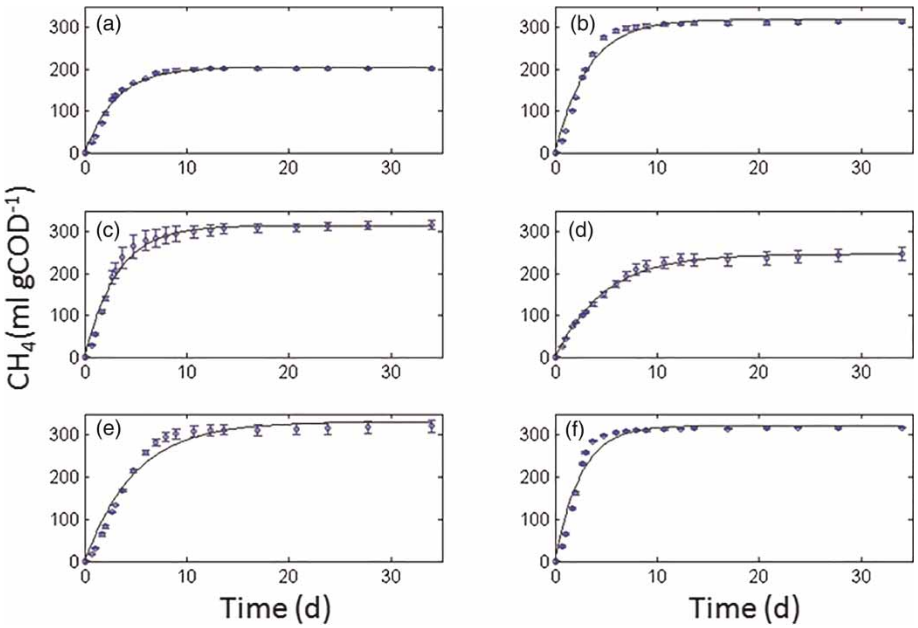

Figure 1 | Cumulative methane production of the fish fractions of tilapia (dots) and the simulated line predicted by the best fit of the first-order model. Error bars: experimental standard deviation between triplicates. (a) scales, (b) skin + fin, (c) viscera, (d) bones, (e) head, and (f) flesh.

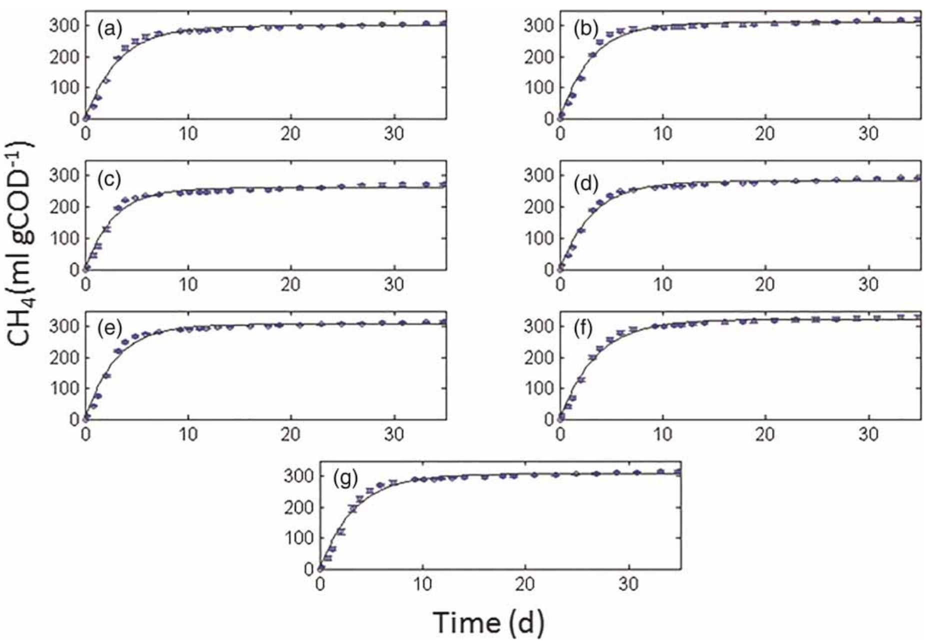

Figure 2 | Cumulative methane production of the fish fractions of sturgeon (dots) and the simulated line predicted by the best fit of the first-order model. Error bars: experimental standard deviation between triplicates. (a) skin bottom, (b) skin back, (c) fin, (d) viscera, (e) bones, (f) head, and (g) flesh.

The parameter values obtained from the parameter estimation procedure are shown in Table 2 for tilapia and sturgeon, respectively. Overall, FR for both species presents a high final biodegradability, for most of the cases above 70%. However, the hydrolysis rate is higher and more constant for the sturgeon fractions than for tilapia, although the flesh of tilapia reached a higher hydrolysis rate than did that of sturgeon.

Table 2 | Kinetic parameter values for the AD of tilapia and sturgeon remaining fractions

	B_0 (mL gVS^{-1})	SD[a]	VC[b]	k_h (d^{-1})	SD	VC	R^2
Tilapia							
Scales	205.13	2.73	1.33	0.33	0.02	4.85	0.99
Skin + fin	319.47	5.93	1.86	0.31	0.02	6.64	0.98
Viscera	315.07	4.96	1.58	0.32	0.02	5.69	0.99
Bones	246.40	2.41	0.98	0.21	0.01	2.97	1.00
Head	331.31	8.95	2.70	0.21	0.02	8.17	0.97
Flesh	322.78	6.81	2.11	0.41	0.03	8.37	0.97
Sturgeon							
Skin bottom	300.69	3.22	1.07	0.30	0.02	5.22	0.99
Skin back	311.69	3.46	1.11	0.33	0.02	5.57	0.98
Fin	261.43	2.92	1.12	0.36	0.02	5.88	0.98
Viscera	283.02	2.66	0.94	0.31	0.01	4.64	0.99
Bones	307.63	3.44	1.12	0.34	0.02	5.71	0.98
Head	323.36	3.12	0.97	0.28	0.01	4.54	0.99
Flesh	307.99	3.40	1.10	0.30	0.02	5.28	0.99

[a]Standard deviation.
[b]Variation coefficient.

In the case of tilapia, the flesh shows the highest hydrolysis rate, which means that hydrolytic biomass can access them in an easier way than the other substrates. Regarding biodegradability, overall, all types of substrates are highly biodegradable except the fishbones. Tilapia bones present the lowest hydrolysis rate (50% less than the highest one) and biodegradability (only 64% of biodegradable organic matter) compared to the other fractions; however, for sturgeon bones, the parameter values are in the same order of magnitude than the other fractions. In the case of sturgeon, the head and the back present the highest level of biodegradability; however, the other fractions are also quite well biodegraded. Likewise, the hydrolysis rates present similar values among the different fractions, with the fin and fishbones being the fastest hydrolyzable substrates.

As mentioned earlier, it is not easy to make a straightforward comparison of the kinetic parameter values since most of the earlier studies have used fish waste as a co-substrate or they have used fish after being pre-treated, for instance after ensiling (Kafle *et al.* 2013). On the other hand, further research is required to determine the influence of the pre-treatment and storage conditions of the solid waste; for instance, grinding and its influence on particle size, as well as freeze drying. Overall, the first-order expression follows the biogas production profiles of all the fractions, although small deviations are observed, probably due to some other phenomena not identified by this simple model, such as nutrient limitation or mass transfer problems.

CONCLUSION

We have presented a complete assessment of the anaerobic biodegradability of the different fractions of two typical fish cultivated in recirculating aquaculture systems, in terms of both ultimate biodegradability and hydrolysis rate. For both fish, the level of biodegradability is high and quite similar; however, the hydrolytic rate tends to vary depending on the fraction and its composition. All the AD profiles can be described as a first-order reaction behavior. The estimated parameters may be used afterward for optimal design and scenario prediction purposes since they can be reliably incorporated in a mechanistic mathematical model of the whole system.

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