Methane Production from Farmed Rainbow Trout Byproducts by Anaerobic Digestion in Morocco

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ABSTRACT

Fish are a staple in human nutrition because of their great nutritional and dietetic value. The processing stages of these fish before being sold generates an important quantity of byproducts (head, viscera, skeleton, fins, scales, tail, and skin). This study aims to determine the methane production, methanogenic potential and the percentage of volatile solids removed by the anaerobic digestion process. The experiment was carried out at mesophilic conditions in a Continuous Stirred-Tank Reactor (CSTR) with a discontinuous feeding mode (batch). The results obtained showed that the byproducts of farmed rainbow trout have a maximum methane production 1176 Nml whose methanogenic potential is 206.68 Nml/gVS with a biodegradability equal to 57.95%. For the kinetic modeling, four models were applied for an adequate fit of the experimental results (first order, MGompertz, transfererence function, and logistic function). The production of methane closest to the experimental results is that estimated by the logistic function with a methanogenic potential of 212.21 Nml/gVS whose correlation coefficient R^2= 0.9870 and a very low percentage of error (1.18%), also the MGompertz model presented results adapted to those of the experiment with a methanogenic potential equal to 223.61 Nml/gVS (R^2= 0.9889 and %error = 2.95); This confirms that these two kinetic models are the most suitable for this type of substrate.

Keywords: anaerobic digestion, farmed fish byproducts, rainbow trout, methanogenic potential, kinetic modeling.

INTRODUCTION

The global fisheries sector produced 179 million tons of fish in 2018, with an annual potential that exceeds 1.5 million tons (FAO 2020), which contributes to food security directly (by increasing the availability and accessibility of food) and indirectly (as a driver of economic development) (MacLeod et al., 2020).

Due to Morocco's advantageous geographic location, which includes a coastline that stretches over 3500 km in both the Atlantic and Mediterranean Seas – a maritime space of about 1.2 million km[2]. The fisheries sector generates an annual production of more than one million tons, making it a structuring resource of the Moroccan economy (Hariri et al., 2017), especially fish farming, which has seen rapid development and expansion in the past ten years. About 70% of fish are processed before being sold (Ivanovs et al., 2018). Depending on the species processed and the type of processing, 20% to 80% of this total is waste (Corkum, 2003). In the circular economy approach these wastes are rich in proteins (70%), fats (18%), carbohydrates (2%) minerals (10%) especially calcium and hydroxyapatite, iodine and selenium (Afilal et al., 2014). They can become byproducts capable of compensating for the scarcity of natural resources and reuse them in several areas such as food processing, cosmetics, agriculture and biogas production as a renewable energy source, used for example to generate electricity, heat or fuel through anaerobic digestion.

Anaerobic digestion (AD) or methanization is a natural system made by a wide variety of microorganisms present in a multitude of environments. It can be observed anywhere in nature where there is organic matter and insufficient oxygen, such as

in marshes and rice fields forming bulbs on the surface of the water and even in the digestive systems of mammals and insects. This degradation produces biogas and digestate. Biogas, which is a product of biological decomposition of organic waste under anaerobic conditions, it is composed of methane (55–65%), carbon dioxide (35–45%), nitrogen (3–6%), hydrogen (0–1%) and hydrogen sulfide (0–1%) (Oke, 2013). Digestate is composed of fertilizer elements used in agriculture after a stabilization or composting step (Eiroa et al., 2012). The process of AD is carried out according to a succession of biological reactions summarized in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig.1)

For more than 40 years, scientific models of anaerobic systems have been developed to increase the efficiency of anaerobic digestion (Ivanovs et al., 2018) and to simulate the production of biogas, in this study four kinetic models were used: First order, MGompertz, Transference and logistic function. The different kinetic parameters were compared with those of the experimental results to deduce the most suitable for this substrate. Currently, no paper has been published on methane production from the mixture of different byproducts of farmed rainbow trout, so the two kinetic models: the transference and the logistic function are not yet used to estimate the methanogenic potential of this type of substrate. Our main objectives were first to study the feasibility of anaerobic digestion of farmed rainbow trout byproducts. Then to calculate the methanogenic

potential as well as the biodegradability, and to compare them by the different results obtained by those of freshwater and marine fish.

MATERIAL AND METHODS

Substrate and inoculum

The substrate used in this study is the byproducts of farmed rainbow trout (fish weighing approximately 804.5 g), they were separated: viscera, head, skeleton, scales and fins and tail (see Fig. 2). They were measured and ground with an electric meat grinder, after which a mixture was formed according to the percentage of each byproduct in relation to the total mass of fish byproducts. This mixture is well homogenized by a food chopper, and then it is placed in the refrigerator at 0°C until its use. The inoculum used was obtained from a wastewater treatment plant. That was stored at mesophilic conditions in the laboratory.

Experimental setup

The AD was carried out in a CSTR digester with a capacity of 1000 ml. The feeding mode is discontinuous (batch). The digester is surrounded by a thermostatic jacket (b) in which hot distilled water (37°C) from the thermostat (c) circulates. It has three openings: The first one is used to feed the digester when the substrate is solid, the second one is used when the substrate is liquid (a)
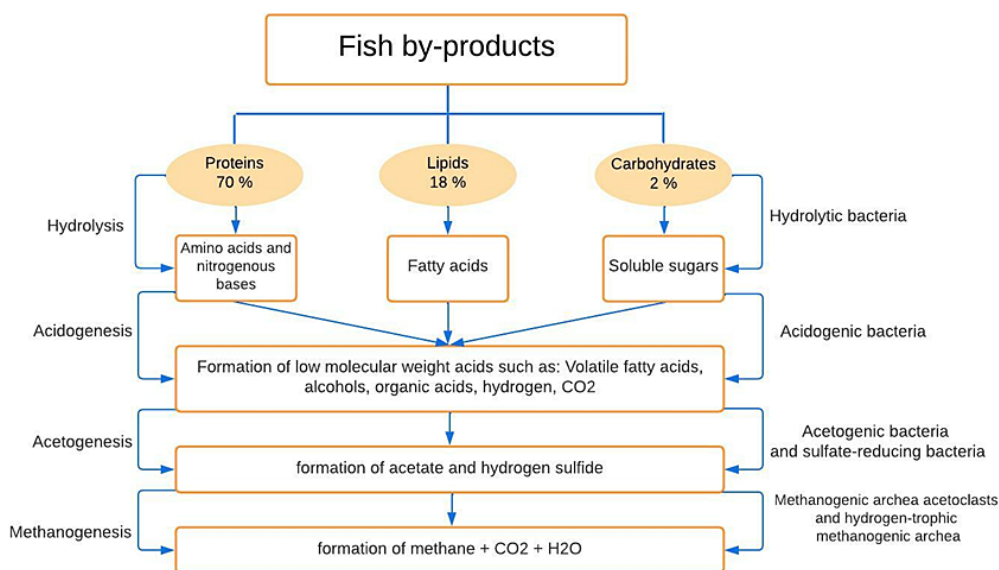

Figure 1. Simplified diagram of the main steps in anaerobic digestion, adapted from (Nges et al., 2012; Gruduls et al., 2018)
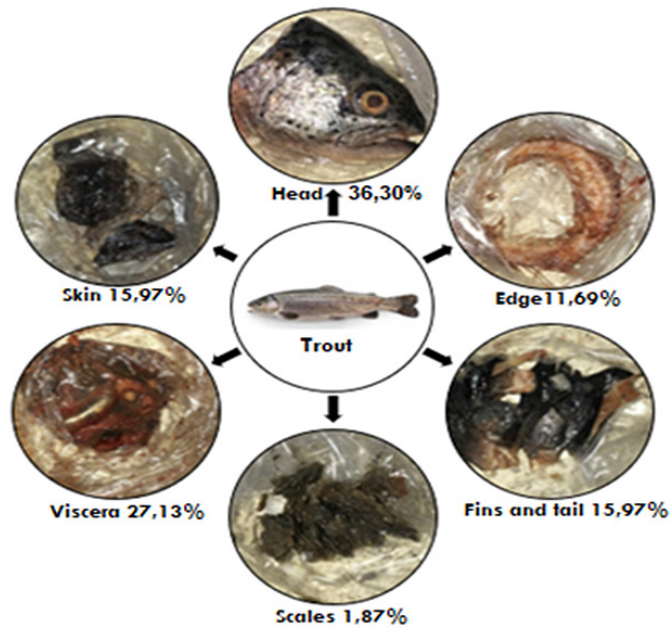
Figure 2. Percentages of byproducts of a farmed fish, trout, in relation to their total mass

and the third one is used to take the sample, in order to carry out the different physico-chemical analyses necessary for the good follow-up of the process. The homogenization of the content of the digester is done continuously with a magnetic stirrer (e). A bubbler (f) containing a solution of sodium hydroxide NaOH (6N) was attached to the digester on one side to absorb the carbon dioxide (CO_2) produced during the anaerobic digestion process, and on the other side by a gasometer filled with water (g). The volume of water moved to the graduated tube (h) is equal to the volume of methane (CH_4) produced.

Analytical methods

Before loading the substrate into the digester, several analyses are necessary to be performed following the protocols of the standard methods of Alpha (Bridgewater et al. 2017). The total solid is measured after drying in the oven at 105°C for 24 hours; for the mineral solid it was obtained after 2 hours of burning the sample in the oven at 550°C. Both analyses are performed three times. The content of calcium carbonate $CaCO_3$ (mg $CaCO_3$/l) and volatile fatty acids (mg CH_3COOH/l) were determined by titration of the two solutions, respectively sulfuric acid H_2SO_4 (0.1 N) and sodium hydroxide NaOH (0.1 N) in the presence of a pH meter calibrated by buffer solutions pH= 4.01 and pH=7.00 and a known volume of the sample. During the six days that the inoculum was degassed, it was twice activated by Gal solution, once at a concentration of 0.5 gVS/l and once at a concentration of 1 gVS/l. The substrate to inoculum ratio was held constant at 2.
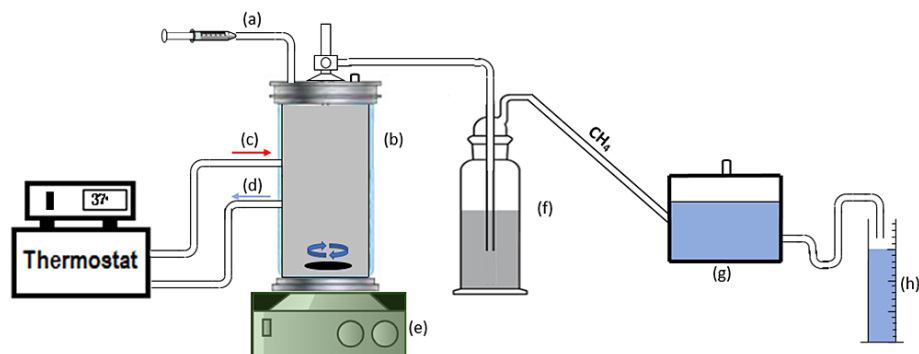

Figure 3. The experimental set-up for anaerobic digestion, (a) feed tube, (b) digester CSTR, (c) hot water inlet, (d) water outlet, (e) magnetic stirrer, (f) bubbler containing NaOH, (g) gasometer, (h) graduated test tube

This ratio was calculated based on the initial volatile solids of substrate and inoculum (Kafle et al., 2012; Helrich, 1990).

$$\frac{S}{I} = \frac{\text{Substrate added (gVS)}}{\text{Inoculum added (gVS)}} \qquad (1)$$

The results are presented in Table 1.

Biodegradability

The biodegradability (BD) is the percentage of organic matter (proteins, lipids, and sugars) that is converted to bioenergy (methane). In this study, biodegradability was calculated from volatile solids removed in gVS/l during the AD process, according to the following Equation:

$$\frac{VSi - VSf}{VSi} X \, 100 \qquad (2)$$

where: *VSi* – initial volatile solid, g/kg; *VSf* – final volatile solid; g/kg.

Kinetic modeling

Kinetic models of AD are complex mathematical models used to estimate biogas and methane production. In this study four models were applied: The first one is the first order model that adjusts the experimental volumes of methane production in time for low substrate concentration. It has been often used for the kinetic characterization of each series of experiments (Borja et al., 1995). It has been described by the following equation:

$$Y = A \, X \, (1 - \exp \, (-K + t)) \qquad (3)$$

(Bakraoui et al., 2019)

This equation can be used to represent a global mass transfer kinetic model for substrate degradation (Redzwan et al., 2004).

The second is the modified Gompertz model, which is the most adequate to describe the role of microbes in the AD process

$$A * \exp(-\exp\left(\frac{\mu e}{A}(\lambda - t) + 1\right)) \qquad (4)$$

(Lahboubi et al., 2022)

The modified Gompertz model may be more appropriate to describe the process AD of fish waste as described by (Kafle et al., 2012) The third is the Transference model, used to fit the AD data, assuming that each process is represented by an input and an output system:

$$Y = \frac{A}{\left[1 + \exp\left(\frac{4\mu \times (\lambda - t)}{A} + 2\right)\right]} \qquad (5)$$

(Redzwan et al., 2004 ; Ourradi et al., 2022)

The fourth is the Logistic model, used to describe the kinetics of bacterial growth and data adjustment, it is a model that is not always valid for all conditions. The equation of its modified form is the following:

$$Y = \frac{A}{\left[1 + \exp\left(\frac{4\mu_m}{A}(\lambda + t) + 2\right)\right]} \qquad (6)$$

Bakraoui et al., 2019 ; Zwietering et al., 1990)

where: *Y* – simulated cumulative methane production in (Nml/gVS), *A* – methanogenic potential in (Nml/gvVS), *K* – Specific constant of the methane produced (h^{-1}), μ – The specific methane production rate (Nml/g VS h), λ – The duration of the lag phase (h), *e* – Exp (1) = 2.7183, *t* – time of digestion (h).

Other kinetic models can be used to simulate methane production from fish waste (Tosun et al., 2004) such as the model of Chen and Hashimoto (Chen et al., 1978; Chen et al., 1980), it is based on fundamental biochemical principles and it gave reliable results for waste containing high total solid (Fongsatitkul et al., 2012) this model is a modification of the Contois mode (Contois, 1959).

RESULTS AND DISCUSSION

Characterization of inoculum and farmed fish byproducts

The physico-chemical characterization of Inoculum and farmed fish byproducts are represented in Table 1.The inoculum used in this study had a total solid (TS = 2.42%) and a volatile solid (VS = 58.43%) that complied with the criteria established by VDI 4630 (Djaafri et al., 2014), which presents the rules and equipment requirements for conducting fermentation tests on organic materials, according to which the amount of substrate should not exceed the amount of inoculum: SV substrate/SV inoculum ≤0.5 (Bücker et al., 2020). T. Edwiges et al, (2018) well detailed the different procedures to maintain the inoculum. The fish byproducts showed average total solid content (ST = 34.41%) and high organic matter content (VS = 88.16%) which is suitable for AD (see Table 1). Table 2 represents a comparative study of different parameters physico-chimical of freshwater and seawater fish byproducts.The analyses of most fish byproducts: Nile perch, Tilapia,

Table 1. Physico-chemical characteristics of the substrate and inoculum

Parameters	pH	TS (F.M.)		VS	VS(TS)	VFA	Alkalinity
Units	-	g/kg	%	g/kg	%	mgCH$_3$COOH/l	mgCaCO$_3$/l
Inoculum	8.17	25.81	2,42	15.17	58.43	960	17500
Substrate	8.69	337.01	34.41	297.10	88.16	1461.11	57344.85

Note: F.M. – fresh matter.

Salmon(heads), Round goby(viscera), Tanzania and Korean sea fish found similar TS and VS values to those found in this study as shown in Table 2 ranging from 31% to 41% total solids (Fonseca et al. 2020; Kafle et al., 2013).

Parameters influencing the stability of anaerobic digestion

The pH is a very interesting indicator in the stabilization and the good progress of anaerobic digestion. It strongly influences the anaerobic digestion process. The process is optimal near neutral pH = 7 (Chen et al., 1978) with an optimal value between 6.5 and 8.5 (Lahboubi et al., 2022) The pH values of the substrate at the beginning and at the end of the process are presented in Table 3. They vary between 8.95 and 8.16 which tells us that the experiment was performed under optimal pH conditions for mesophilic anaerobic digestion. Other parameters have a direct impact on the stability of

the process in batch digesters, such as the alkalinity and the VFA/ALK ratio, in this study it was found that the alkalinity of the digester content increased from 1476 to 2325 mg CaCO$_3$/l and the values of volatile fatty acids decreased from 990 to 480 mg CH$_3$COOH/l, also the ratio VFA/ALC equal to 0.20 which confirms the low concentration of VFA and therefore the absence of inhibition of methanogenic bacteria (Raposo et al. 2009), a VFA/ALK ratio of about 0.70 results in the destabilization of the process (Islam et al., 2012 ; Kafle et al., 2012). For the inoculum, the alkalinity (17500 mg CH$_3$Ca/l) and pH (8.17) were high, so there was no need to add external compounds such as CaCO$_3$, NaCO$_2$ and NaOH to maintain them (Kafle and Kim, 2012).

Cumulative methane production

The methane production rate is the ratio of the volume of methane produced per unit time. The methane production rate and the cumulative

Table 2. Comparison of the physical-chemical properties of freshwater and seawater fish byproducts with literature

Parameters		pH	TS		VS	VS(TS)	VFA	Alkalinity	References
Units		-	g/kg	%	g/kg	%	mg CH$_3$COOH/l	mg CaCO$_3$/l	-
Trout [1] (Byproduct mix)		8.69	337.01	34.41	297.10	88.16	1461.11	57344.85	This study
The viscera of the trout [1]		ND	629	ND	989	ND	ND	ND	(Albuzio et al., 2011)
Nile Perch [1]		7.10	ND	37.4	ND	82.37	121060	5230	(Kassuwi et al., 2012)
Tilapia[1]		ND	ND	34.3	ND	83.4	ND	ND	(Fonseca et al., 2020)
Carp common [1] (DM)		ND	ND	29.1	ND	89	ND	ND	(Bücker et al., 2020)
Mix of red snapper, corvine and tuna [2] (DM)		7.4	ND	25.2	ND	88.9	1515	650	(Escobar, 2019)
Raw fish waste (market in Korea)		ND	ND	31.30	ND	27.25	ND	ND	(Kafle et al., 2013)
Befor to digestion		8.16	ND	ND	ND	ND	906	9570	
After digestion		7.79	ND	ND	ND	ND	720	10650	
Salmon heads [1]		ND	ND	41.2	ND	ND	ND	ND	(Nges et al., 2012)
Round goby 2	Head + Skin OS intestines	ND	ND	20.5 22.2 36.7	ND	76.5 75.3 82.6	ND	ND	(Gruduls et al., 2018)
Fish byproduct in Tanzania 2		6.9	ND	32.2	ND	55.3	ND	2280	(Mshandet et al., 2004)

Note: ND – not determined, DM – dry matter, 1 – freshwater fish, 2 – seawater fish.

Table 3. Physico-chemical results of before and after AD of trout byproducts

Parameters	pH	TS (MF)		VS	VS (TS)	VFA	Alkalinity
Units	-	g/kg	%	g/kg	%	mg CH_3COOH/l	mg $CaCO_3$/l
Before digestion	8.95	28.43	3.02	19.32	67.94	990	1476
After digestion	8.16	15.45	1.66	8.12	52.52	480	2325

methane production are presented in Figure 4. Six phases can be seen in the curve representing the cumulative production of methane:

- phase 1 – this is the latency phase, the bacteria adjust to their new environment during this phase, which lasts a few minutes to a few hours following the addition of the substrate (Beniche, 2021); after 4 hours of digestion in the reactor, there was a rate of methane production of 21 Nml CH_4;
- phase 2 – during this phase, methane production slows down. As early as the first day (after 20 hours), the rate of synthesis fell from 26 Nml CH_4 to 11 Nml CH_4 after 44 hours of digestion; this decline may have been caused by the rise in pH (Antonio, 2011; Albuzio et al., 2011);
- phase 3 – from day 4 (116 hours) to day 8 (188 hours), the rate of methane production rose significantly due to the bacteria's ability to produce all the enzymes required for the breakdown of organic materials; The rate of production increased to 77 Nml CH_4;
- phase 4 – the rate of methane production decreased rapidly until day 12 (308 h), after which a small increase was observed. The reason for the low methane production from day 2 to day 4 and day 8 to day 12 may be due to the decomposition process taking place in the fish byproducts due to the high protein (up to 70%) and fat (up to 18%) content (Afilal et al., 2014; Ghaly et al. 2013). The conversion of carbohydrates (2%) (Afilal et al., 2014; Ghaly et al. 2013) occurs rapidly (a few days) but proteins, and fats may require a few weeks (Kafle et al., 2012);
- phase 5 – methane production has been continuously dropping because the bacteria have limited access to organic materials;
- phase 6 – Because of substrate exhaustion, methane synthesis has ceased.

In this study, the methanogenic potential of trout byproducts in the mesophilic AD was 206.68 NmL/gVS. Assuming the farm produces 50 tons of waste per year, 3073.5 m³ of methane could be generated annually

Biodegradability

Table 4 presents Comparative study of anaerobic digestion results for different fish byproducts. The percentage of volatile solids removal under experimental conditions in this study was significant (57.95%). Fish byproducts obtained from the Korean Sea presented a high percentage of VS removed (77%) as shown in Table 4. Since these two substrates have almost the same chemical composition (rich in proteins and lipids), two different results can be explained by the types of microbial species present in the inoculum used in each study, and also the cell density of these microbial species (Thouand et al., 2011). Several studies have been established to increase the percentage of VS removed and therefore methane production, such as changing the ratio (tuna, sardine, mackerel), codigestion (Round goby, tuna), and also different types of pretreatments have been used, especially the thermal and enzymatic pretreatment (Nile Perch, salmon head) see Table 4.

Kinetic modeling

To analyze and predict methane production from farmed rainbow trout byproducts and compare it with experimentally produced methane, several kinetic models were used (MGompertz, Logistic, Transference and First Order), the different results are presented in Table 5.

The simulated methanogenic potential closest to the experimental results (212,23 N ml/g VS) was found using the Logistic model with a better value of correlation coefficient R^2 ($R^2 = 0.9870$) and a very low % of error (1.18%). The MGompertz model also presented results close to the experiment by a methanogenic potential equal to 223, 61Nml/g VS and an R^2 value close to that of the Logistic ($R^2 = 0.9889$) as well as for the percentage of error (2.95%), these results are different to those found in the literature (Kafle et al., 2012) whose experimental methane yield was 554 ml/gVS and that estimated by the MGompertz model was 455,06 ml/gVS (deduced from the study of (Kafl et al., 2012) with R^2 equal to
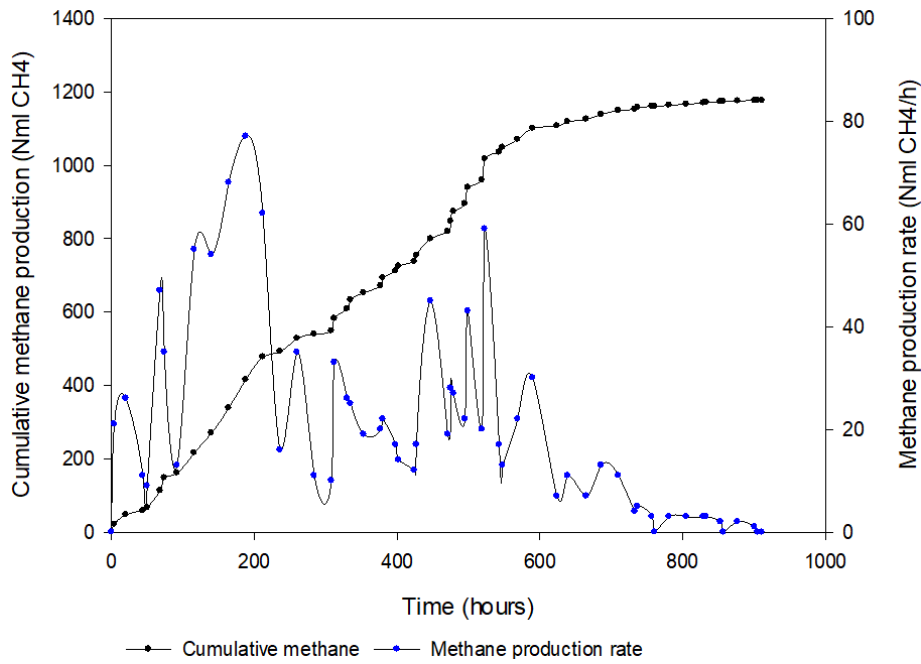
Figure 4. Evolution of cumulative methane production and methane production rate as function of time

Table 4. Comparative study of anaerobic digestion results for different fish byproducts

By products of different fish	VS removal (%)	Methanogenic potential	Conditions	Retention time (d)	Reference
Trout byproducts	57.95	206.68 $NmlCH_4$/g VS	Mono-digestion	38	This study
The viscera of the trout	ND	474 $NmlCH_4$/g VS	Mono-digestion	27	(Albuzio et al., 2011)
Nile Perch	ND	500–610 CH_4ml/g VS	Co-digestion; Thermal and enzymatic pretreatment	42	(Kassuwi et al., 2012)
Tilapia	ND	445 $NmlCH_4$/g VS	Mono-digestion	38	(Fonseca et al., 2020)
Common carp (DM)	ND	540 $mlCH_4$/ g VS	Thermal and enzymatic pretreatment	19	(Bücker et al., 2020)
Mixture of red snapper, corvine and tuna fish (DM)	ND	460 $mlCH_4$/g VS	Dilution based on total solid	28	(Escobar, 2019)
Fish waste (market in Korea)	77	554 $mlCH_4$/g VS	Mono-digestion	60	(Kafle et al., 2013)
Salmon heads	ND	828 $mlCH_4$/g VS	Co-digestion; Thermal and enzymatic pretreatment	33	(Nges et al.,2012)
Round goby (Mix of head, bone, skin, intestine)	ND	639 $mlCH_4$/g VS	Co-digestion	31	(Gruduls et al., 2018)
Tuna	66–81	180–280 $mlCH_4$/g VS	Change in ratio (VSs/VSi): 1.2 ;3.1 ;6.2	77	(Eiroa et al., 2012)
Tuna	38–74	160–210 $mlCH_4$/g VS	Co-digestion	56	(Eiroa et al., 2012)
Sardine	62–74	200–250 $mlCH_4$/g VS	Change in ratio (VSs/VSi): 1.1 ;2.8 ;5.7	77	(Eiroa et al., 2012)
Mackerel	49–84	40–350 $mlCH_4$/g VS	Change in ratio (VSs/VSi): 1.3;3.3;6.5	77	(Eiroa et al., 2012)
Needle	61–74	40–260 $mlCH_4$/g VS	Change in ratio (VSs/VSi): 1.2;3.1;6.2	77	(Eiroa et al., 2012)
Byproducts of fish in Tanzania (offal, scales, gills and wash water)	ND	390 $mlCH_4$/g VS	Co-digestion	ND	(Mshandete et al., 2004)

0,904 and % error equal to 2,4. In another study of freshwater fish Tilapia, the modified Gomeprtz resulted a simulated methanogenic potential of 438 Nml/gVS while the measured methanogenic potential was 445 Nml/gVS (difference 1.6%) and the R^2 was 0.994 (Fonseca et al., 2020)

For the two kinetic models: Transference and First order, they have presented different methanogenic potentials (MP) from the experimental (300.76 and 300.29 NmL/gVS, respectively), regarding the kinetic parameters (R^2 and % error) are almost similar for both models in this study. The First order model in the literature(Kafle et al., 2012) presented different results from the experimental (the measured yield was 554 ml/g VS and the simulated yield was 381.40 ml/g VS) including R^2 equal to 0.751 with a percentage error of 0.010%. On the other hand, the First order model presented results very close to those of the experimental, according to Bucker et al. (2020) the measured methanogenic potential was 445 Nml/gVS and the estimated one was 444 Nml/gVS (difference of 0.2%) with $R^2 = 0.997$.

Concerning the lag phase, it is important in this study for the Logistic model ($\lambda = 53.48$ h) also for MGompertz ($\lambda = 31.18$ h), and almost negligible for the Transference model (0.98 h), in the literature it was very high ($\lambda = 14.1$ day) using the MGompertz model according to Kafle et al. (2012) and almost the same as this study ($\lambda = 1.8$ day) using the MGompertz model according to Fonseca et al. (2020). For the parameter μ, which represents the rate of methane production per unit time (hour) it was observed that the three models MGompertz, Logistic and transference had almost similar values (0.37, 0.38 and 0.44 Nml/gVS, respectively)

According to the findings of the kinetic modeling utilized in this study, the two models MGompertz and Logistic are the most suitable to the experimental results and, as a result, to calculating the kinetic parameters of the anaerobic digestion of the byproducts of farmed fish (trout). The two other models, First order and Transference, on the other hand, were not suggested for the byproducts of farmed fish.

Table 5. Kinetic models used in this study

Models	MP (Nml/g VS)	A (Nml/g VS)	μ (Nml/g VS .h)	λ (h)	K(h⁻¹)	R^2	Error (%)
MGompertz	206.68	223.61	0.37	31.18	-	0.9889	2.95
Logistic	206.68	212.21	0.38	53.84	-	0.98701	1.18
Transference	206.68	300.76	0.44	0.98	-	0.9825	6.97
First order	206.68	300.29	-	_	0.0146	0.9825	6.95

Note: MP – Methanogenic potential, *A* – Results estimated by the different models.
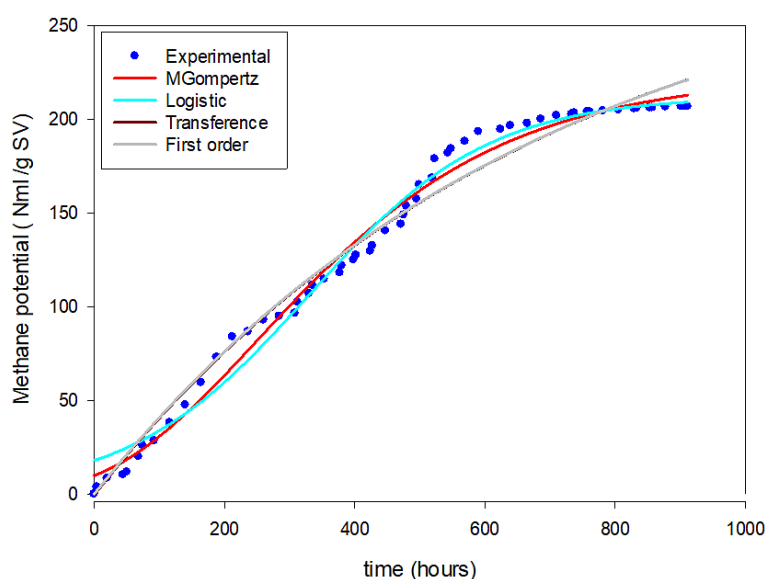

Figure 5. Evolution of methane potential as a function of time during mesophilic anaerobic digestion (batch study)

CONCLUSIONS

Generally, the studies made on organic waste have evaluated either their polluting potential or their harmful effects on human health and the environment. Thanks to the process of anaerobic digestion, it has been possible to see these biodegradable wastes on the positive side and to consider them as a source of renewable energy "biogas". This process has provided a digestate that can be used in place of fertilizers and chemical fertilizers.

This study showed that the mixture of different byproducts of farmed rainbow trout has a high organic load VS(TS) of 88.16% which was converted to methane with a methanogenic potential equal to 206.68 Nml CH_4/gVS with a biodegradability of 57.95%. The MGompertz model and the logistic function are the most efficient kinetic models for simulating the methanogenic potential of rainbow trout by-products among the four models used in this study. These positive findings provide new opportunities for academics to carry out more research to enhance methane production as well as to deepen the kinetic study, which is rarely carried out for this kind of substrate.

Acknowledgements

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