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1 **BIOMETHANE FROM FISH WASTE AS A SOURCE OF RENEWABLE ENERGY**
2 **FOR ARTISANAL FISHING COMMUNITIES**

3
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10
11 **ABSTRACT**
12 The potential of biogas production from fish waste as source of renewable energy for fishermen
13 communities was evaluated. Four different fish waste concentrations (1%, 1.5%, 2% and 2.5%
14 total solids (TS)) were digested during 28 days at mesophilic conditions. Biochemical Methane
15 Potential (BMP), volatile fatty acids (VFA) concentration and ammonia concentration were
16 analysed during the experiment. Energy production and economic projections were performed to
17 estimate the number of families that can benefit from the biogas production in Tumaco, Colombia.
18 The 1% TS had the highest BMP (464.5 mL CH₄/g VS) and the lowest VFA production (2515
19 mg/L); in contrast, the 2.5% TS had the highest VFA production (11302 mg/L) and the lowest

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20 methane production (206.86 mL CH₄/g VS). The treatments with 1.5%, 2% and 2.5% TS exhibited
21 diauxic growth as result of different solubilisation rates in the fish waste components. The
22 energetic and economic analyses estimated a yearly energy production of 489 MWh, which can
23 satisfy the electric energy consumption or the cooking energy demand of 230 fishermen families.
24 The results showed that biogas production from fish waste is a viable and sustainable alternative
25 to adequately manage this material and provide renewable energy to fishermen communities.

26

27 **Key words:** Anaerobic digestion; fish waste; biogas; renewable energy; artisanal fishers.

28

29 **1 INTRODUCTION**

30 Colombia produces approximately 230.000 tons of fish every year, from which 80.000 tons are
31 from fish farming and 150.000 tons from industrial and artisanal fishing [1]. Artisanal fishing
32 includes the small scale, low technology and low capital fishing practices performed by fishing
33 households. Colombia has between 67.000 and 150.000 artisanal fishers [2] which are
34 responsible for supplying 75% of the whole Colombian fish market [3]. Tumaco, the second largest
35 city on the Colombian Pacific coast, is one of the most dynamic artisanal fishing centres. Tumaco
36 produces more than 1.000 tons of fish per year and has an artisanal fishing production that
37 accounts for 9% of the production of artisanal fisheries in the country [4]. As a zone affected by
38 the Colombian armed conflict, Tumaco and its population are under persistent social, economic
39 and environmental issues.

40

41 One of the main environmental and economic issues affecting artisanal fishers is produced by fish
42 waste (FW). Artisanal fishermen produce FW mainly from traditional practices during fish catching

43 and processing. During fish catching, the viscera are thrown directly into the sea during prolonged
44 fishing operations. whereas in fish processing, generally at the commercialisation sites, skin,
45 bones and other residues are produced and disposed into the ocean or landfills. It is estimated
46 that around 45% of the total fish production corresponds to fish waste (FW) [5], representing
47 significant economic losses for this sector. Besides the economic losses, inadequate FW disposal
48 generates high loads of organic matter with high degradation potential, which affects aquatic
49 ecosystems, produces greenhouse gases and bad odours [6]. This, in turn, favours the
50 proliferation of vectors and the transmission of intestinal diseases (dysentery, diarrhoea and
51 gastritis) and endemic diseases (malaria, yellow fever and dengue) [7] endangering the
52 communities in Tumaco.

53

54 Currently, FW from industrial fishing is used for low-value animal feed products, through the
55 production of fishmeal, silage and fertilizers [8]. In recent years, Anaerobic Digestion (AD) appears
56 as a promising technology for the environmentally safe transformation and management of FW
57 [9-11]. Generally, AD is defined as a biological process carried out in absence of oxygen where
58 the biomass is degraded and stabilised by a microbial consortium to produce methane, a product
59 used as a source of renewable energy. Besides methane other authors have reported the
60 transformation of FW into other added-value products, such as volatile fatty acids, oils and proteins
61 [8, 12, 13].

62 FW is a heterogeneous waste rich in proteins and lipids compounds that facilitates the production
63 of high methane yields [14]. But at the same time, FW releases components such as free long
64 chain fatty acids, high amounts of ammonia and high concentrations of light metals such as
65 calcium, sodium, potassium and magnesium, that affects the anaerobic degradation [15].
66 Biomethane production from fish waste has been evaluated in direct digestion and in co-digestion
67 with other wastes. Biomethane using exclusively FW has been produced from residues such as

68 gilthead seabream FW sludge ($361 \text{ Nm}^3 \text{ CH}_4 \text{ Mg VS}^{-1}$) [11], saline fish waste ($428 \text{ mmol CH}_4 \text{ kg}$
69 $\text{COD}^{-1} \text{ day}^{-1}$) [16], offal fish waste ($164.7 \text{ L CH}_4 \text{ kg COD}^{-1}$), fish canning facilities (0.59 g COD-
70 $\text{CH}_4 \text{ g COD}^{-1}$ and $441\text{--}482 \text{ mL g VS}^{-1}$) [12, 17] and sturgeon heads and viscera ($1.4 \text{ L CH}_4 \text{ g}$
71 VS^{-1}), [18], [19]. FW co-digestion has produce biomethane from mixtures of strawberry and FW
72 ($0.205 \text{ m}^3 \text{ CH}_4 \text{ kg VS}^{-1}$) [20], FW and bagasse ($409.5 \text{ mL g VS}^{-1}$) [21], FW and sisal pulp (0.62 L
73 $\text{CH}_4 \text{ g VS}^{-1}$) [22] and FW and cow manure ($0.4 \text{ L CH}_4/\text{g VS}$) [23, 24]. The previous research in
74 biomethane production from FW evidenced the need of an inoculum adapted to low C:N ratios, a
75 positive effect by adding nutrient rich materials and variable methane production depending of the
76 FW type. However, the production of methane from FW has not focused in fish wastes from
77 artisanal fishers and the further application of this waste to supply the energy requirements for in-
78 need communities such as Tumaco's artisanal fishermen households. Artisanal FW has higher
79 solids and nitrogen concentrations than industrial FW, these differences can generate differences
80 in the retention times, biomethane production and nitrogen concentrations than previously
81 reported FW.

82

83 Therefore, to solve the energy and waste management requirements in the fisherman
84 communities in Tumaco, Colombia, the aim of this study was to evaluate the production of
85 renewable energy, in the form of biogas, from artisanal fish wastes from Tumaco, Colombia and
86 to estimate the energy production and the number of families benefited by transforming Tumaco's
87 fish waste into biogas.

88

89 **2 MATERIALS AND METHODS**

90 **2.1 Inoculum and substrate**

91 The inoculum was a methanogenic inoculum (MI) obtained from an upflow anaerobic sludge
92 blanket (UASB) reactor treating wastewaters from the slaughterhouse plant of *Carnes y Derivados*
93 *de Occidente SA*, (Candelaria, Colombia). The FW was collected from artisanal fishers from the
94 port of Tumaco (Nariño, Colombia) and consisted principally in guts, digestive tracts and viscera
95 from a mixture of different types of fish species including red snapper, corvine and tuna. After
96 collection, FW was transported to the laboratory in a 4 °C refrigerated container. The
97 transportation process between collection and cold storage in the laboratory had a duration of 4
98 hours. In the laboratory, the samples were ground to 5 mm particle size using a food processor
99 (Black & Decker FP1336) and then frozen until use.

100

101 **2.2 Experimental set-up**

102 In order to determine the biogas production, the standardised Biochemical Methane Potential
103 (BMP) test was carried out according to Owen et al. (1979) [25] and Angelidaki et al. (2009) [26].
104 Duran bottles of 250 mL with an effective volume of 210 mL and rubber stoppers provided with a
105 valve for methane measurement were used. The effect of FW concentration on methane
106 production followed a completely randomised experimental design with four levels and three
107 replicates. The four FW total solid (TS) concentrations were 1%, 1.5%, 2% and 2.5% TS. The
108 experiments were carried out using an FW to inoculum ratio of 1:1, in terms of Volatile Solids (VS)
109 during 28 days at 37±2 °C, without pH control and with manual agitation once a day. Methane
110 production, Volatile fatty acid (VFA) concentration and ammonia concentration were used as
111 response variables. Methane production was measured daily and the methane volumes were
112 corrected by subtracting the mean methane volume of the inoculum control and were converted
113 to standard temperature and pressure (STP, 0°C and 760 mm Hg). VFA and ammonia were
114 measured from 5 mL samples procured from the experimental units at days 1, 7, 14, 21 and 28.
115 Then, the samples were centrifuged at 3000 rpm for 20 minutes (centrifuge brand) and finally

116 refrigerated at 4°C until analysis. The experiment included two bottles with inoculum and without
117 FW as control. The BMP results at day 28th were analysed in a one-way ANOVA and the Duncan's
118 means tests. The statistical analysis was performed with the GLM procedure of the SAS Software
119 University Edition.

120

121 **2.3 Analytical methods**

122 The inoculum and substrate characterisation, as well as the analysis of total VFA and ammonia
123 were carried out following the protocols of the APHA standardised methods [27]. Total solids (TS)
124 dried at 103-105 °C (method 2540 B), Fixed and volatile solids (VS) ignited at 550 °C (method
125 2540 E), Titrimetric method for ammonia (method 4500-NH₃ C). Distillation method for VFA
126 (method 5560 C). Titrimetric method for alkalinity (Method 2320 B) and Closed reflux, colorimetric
127 method for COD (method 5220 D). The production of VFA and ammonia was determined with a
128 Kjeflex K-360 Buchi distiller. The carbon and nitrogen contents of the inoculum and FW were
129 determined using the ASTM D 5373 standard from 2014.

130

131 The daily biogas production was measured with the water displacement technique using a Mariotte
132 bottle. In selected samples, the methane and carbon dioxide content were analysed using gas
133 chromatography ((GC) *Agilent Technologies 6890^a* with a Hewlett Packard 7694E static
134 headspace device, thermal conductivity detector and flame ionisation detector (S-HS/GC
135 /TCD/FID)). The chromatographic analysis was performed with a monolithic carbon column (30 m
136 x 0.53 mm x 3 µm) and a zeolite (30 m x 0.53 mm x 50 µm) column.

137

138 **3 RESULTS AND DISCUSSION**

139 **3.1 Physicochemical characterisation of MI and FW**

140 **Table 1** describes the characterisation of the FW collected from the artisanal fishers in Tumaco
141 and the Methanogenic inoculum from the UASB reactor from the slaughterhouse plant. FW had a
142 mixture of liquid and solid compounds (25.2% TS) with high organic matter content (88.9% VS in
143 dry basis) and high nitrogen content (8.84% TKN in dry basis). Tumaco's artisanal FW had similar
144 characteristic as FW reported from fish canning facilities which had TS% between 20% and 40%
145 and VS% between 73% and 95% [12, 19, 20]. FW from fish farms doubled the amount of total
146 solids (50%) compared with Artisanal FW and Fish canning wastes, however, fish farms FW has
147 a similar VS% as artisanal FW (88-90%) [17]. The significant amounts of biodegradable materials
148 in the artisanal FW have been associated with the production of biogas from FW in other
149 investigations [12, 19]. The majority of biodegradable materials in FW are lipids (5–55%) proteins
150 (40–75%) and carbohydrates (5–13%) [23, 28], materials successfully transformed into biogas
151 using anaerobic digestion; although, the high protein and lipids content can reduce the methane
152 yield. The methanogenic inoculum had a high VS (84.9 % in dry basis) and high nitrogen content
153 (8,9 % NTK in dry base) reflecting a significant amount of microbial biomass and residual nitrogen
154 from the UASB reactor employed for treating slaughterhouse wastes. The considerable amount
155 of ammonia in the artisanal FW and the MI conferred the fermentation broth with a high buffer
156 capacity. The low C/N ratio of the substrate and the inoculum is similar to the values observed in
157 previous biomethane from fish waste processes and it was associated with the production of
158 higher concentrations of ammonia during digestion.

159

160 **3.2 Anaerobic digestion of artisanal Fish Waste**

161 **3.2.1 Methane production from artisanal FW**

162 Methane production from artisanal FW (cumulative and production rate) is depicted in **Figure 1**.
163 All the artisanal FW concentrations had a high methane yield during days 1 and 2 of the
164 experiment. This initial peak was the highest methane production rate observed in all the
165 treatments. The 1.5, 2 and 2.5% TS treatments reached the highest rate (43.58, 75.5, 43.14 mL
166 $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$) during the first day; whereas, 1% TS treatment reached the highest production
167 rate during the second day (48.5 mL $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$). In all cases, on day 3, the methane production
168 reduced between 50 and 100%. After this, the 1% treatment kept its methane production between
169 12 and 25 mL $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$ until day 20, when the methane production started to decrease. In
170 contrast to 1%TS treatment, the treatments with 1.5, 2 and 2.5% TS evidenced a constant but low
171 production of methane after day 3. The duration of this constant phase was dependent of the FW
172 concentration (1.5% 10 days, 2.0% 13 days, 2.5% 20 days) and was followed by an increment in
173 the methane production or a constant methane production until the end of the experiment (**Figure**
174 **1**). After the initial methane production peak, in average, the lowest methane production rate was
175 observed on the concentration with 2.5% TS (6 mL $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$), followed by 2% TS (12 mL
176 $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$) and 1.5 and 1 % TS (14 mL $\text{CH}_4 \text{ g VS}^{-1} \text{ d}^{-1}$). Although, the methane production
177 rate of the 1% TS treatment was higher in the initial days, it was lower than that of 1.5% TS
178 treatment at the end of the fermentation process. The presence of a high production peak in the
179 initial days has been reported in FW from canning facilities and fish farming and has been
180 associated with the transformation of substrates that were easy to digest from the FW or the
181 inoculum [17, 19]. Similar to artisanal FW, a second increment in the methane production rate was
182 also reported in the production of biomethane from mackerel fish waste and cuttlefish waste [19].
183 The methane production inhibition observed in the high concentrations (2.5 and 2 % TS) were
184 also described in fish canning wastes (2.5 and 5% TS), FW and strawberry wastes co-digestion,
185 and FW and cow manure co-digestion (19% FW) [12, 20, 23]. Besides FW initial load, the FW to
186 inoculum ratio (F/I) has been a factor influencing methane production from FW. FW and sisal pulp
187 co-digestion, fish canning wastes and fish farm wastes reached the highest yields using an F/I of

188 0.5. Similar to Artisanal FW, F/I ratios of 1 had better performances in FW from 4 different types
189 of fish [12]. The different results in the FW literature can be explained by the differences in
190 temperature, the F/I ratio and the intrinsic differences associated with the fish species. The species
191 from which the fishing residue comes is also a factor to be taken into account, since Eiroa *et al.*,
192 (2012) [12] reported methane potentials of: 0.25 L CH₄/g SV_{added} for sardine waste, 0.26 L CH₄/g
193 SV_{added} for needle fish waste, 0.28 L CH₄/g SV added for tuna waste and 0.35 L CH₄/g SV_{added} for
194 mackerel waste.

195

196 The cumulative methane production profile was different in all the treatments. The 1 % TS
197 treatment exhibited a unique production phase achieving a BMP value of 464.5 mL CH₄/g VS. In
198 contrast, the concentrations of 1.5%, 2% and 2.5% TS exhibited two methane production phases
199 during the experiment producing a total BMP of 452.6 mL CH₄/g VS, 425.2 mL CH₄/g VS and
200 206.9 mL CH₄/g VS, for the TS concentrations of 1.5, 2 and 2.5%, respectively. The presence of
201 a second phase evidenced that the fermentation broth has an easier biodegradable fraction and
202 a more complex fraction that requires a longer period of hydrolysis, resulting in diauxic growth
203 [29]. In FW, proteins have a relatively high solubilisation rate, while that of lipids is relatively low
204 [30]. In fact, during the first days of the experiments, an immiscible layer of fat and its degradation
205 products was observed in the upper part of the reactors. The disappearance of this phase was a
206 proof of the FW heterogeneity producing diauxic growth in the reactor. Diauxic growth behaviour
207 has been observed in the methane production from pacific saury fish waste, mackerel fish waste
208 and cuttlefish waste, however, these fermentations had a longer duration than the process
209 reported in this article with artisanal fish wastes [19]. Similar to FW, the AD of other heterogeneous
210 substrates had evidenced diauxic growth, for example, the digestion of wastewater screenings
211 [31]. In the future, to improve the production of biogas from artisanal FW is necessary to evaluate
212 economic methods to achieve rapid solubilisation and hydrolysis of fats. These methods will help

213 to avoid the accumulation of long-chain fatty acids (LCFA). LCFA can accumulate in reactors
214 affecting metabolic transport and generating methane production inhibition [32].

215

216 A one way ANOVA was performed using the BMP results from day 28. The ANOVA evidenced
217 the presence of significant differences among the treatments with a *p-value* below the alpha
218 ($0.05 > 0.0010$) (Supplementary Material). As the ANOVA proved differences among the
219 treatments, the Duncan's means test was used to see how different the treatments were (**Figure**
220 **2**). The treatments with 1 and 1.5% did not have significant differences; however, they were
221 different from the treatments with 2 and 2.5%. The treatments with 2 and 2.5% were also different
222 between each other. This indicates that it is possible to use a TS value of 1 or 1.5% to produce
223 similar amounts of BMP, however, 1% TS reached the maximum production faster than the others
224 concentrations.

225

226 3.2.2 *Volatile fatty acids and ammonia production from artisanal FW*

227 The ammonia concentration during the AD of Tumaco's artisanal FW is depicted in **Figure 3**. The
228 ammonia concentration profiles were opposite to methane production, as the treatments with the
229 highest initial FW concentration exhibited the highest ammonia concentrations. The highest
230 amounts of ammonia observed in each treatment were 943, 730, 542, and 431 mg NH₄⁺ L⁻¹ for
231 the 2.5, 2, 1.5 and 1 % TS treatments, respectively. In all treatments, the ammonia had the most
232 significant increment during the first week of digestion and was associated with the steep reduction
233 in methane production observed after the third day of the digestion (**Figure 1**). After this initial
234 increment, the ammonia concentration was almost constant for the treatments with 1, 1.5 and 2
235 % TS. The 2.5% concentration experienced a second increment in the ammonia concentration
236 during the 21st day of digestion. This increment was also associated with a reduction in methane

237 production (**Figure 1**). The lowest methane production observed in the 2.5% ST can be explained
238 by the high ammonia concentrations (between 705 and 944 mg/L) during the whole process
239 (**Figure 3**). The anaerobic digestion of wastes with high concentrations of solids has been
240 inhibited by the accumulation of ammonia released by protein hydrolysis [15, 33]. In fact, it has
241 been reported that ammonia concentrations greater than 800 mg L⁻¹ are inhibitory for anaerobic
242 processes. In alkaline conditions, ammonia is in its free form and easily permeates the bacterial
243 cell wall, causing a toxic effect [32, 34].

244

245 The anaerobic digestion of artisanal FW achieved the production of significant amounts of VFA
246 (**Figure 4**). All FW concentrations reached a VFA production above 2.000 mg L⁻¹. The 2.5% TS
247 treatment obtained the highest VFA concentration (11.302 mg L⁻¹) followed by the TS
248 concentrations of 2%, 1.5% and 1% TS with VFA concentrations up to 8.918 mg L⁻¹, 5.090 mg L⁻¹
249 and 2.515 mg L⁻¹, respectively. Similar to ammonia concentration, VFA concentration was
250 opposite to methane production as the treatments with the highest initial load produced the highest
251 VFA concentration. In all FW concentration, the VFA production had the most significant increment
252 in the initial 7 days; however, the maximum VFA concentration was reached after 14 days of
253 digestion. The high VFA production during the initial days related to the reduction in methane
254 production. The ammonia and VFA concentrations caused a reduction in the BMP of the highest
255 TS concentrations. High protein wastes produce excessive amounts of ammonia and VFA as the
256 anaerobic degradation of amino acids follows the Stickland's reaction and the two mayor products
257 from this reaction are ammonia and VFA. In methane production excessive VFA accumulation
258 produces a decrease in the pH and an affectation of methanogenic microorganisms [15]. Non-
259 ionised VFA inhibit methanogenic activity, when acid production rates exceeds the consumption
260 rates. The VFA concentration achieved by the artisanal FW with 2.5% concentration was higher
261 than other FW at the same concentration, the difference in VFA production between artisanal and

262 fish wastes is associated to the higher nitrogen concentration observed in artisanal FW than other
263 FW reported in the literature. In other substrates, higher nitrogen concentration is associated with
264 the production of higher amounts of VFA [35]. The production of high VFA concentrations
265 evidence the potential of artisanal FW as raw material for the production of VFA similar to other
266 high protein wastes such as slaughterhouse blood [35, 36].

267

268 **3.3 Energy and economic projection in the city of Tumaco**

269 The results from this research evidenced the potential for using artisanal FW as a source for
270 methane production. Renewable energy sources are fundamental for the communities in Tumaco
271 because the groups outside the law frequently attack the electric infrastructure generating lack of
272 electricity to power Tumaco's population. **Table 2** summarise the economic analyses performed
273 in the article. Between March and December of 2017, the artisanal fish landed in Tumaco was
274 942.4 tons [4]. Assuming a monthly fish landing average of 94.24 tons, for the whole year, the fish
275 landed would reach approximately 1.131 tons. Therefore, considering that around 45% of the
276 landed fish becomes FW [5]. Tumaco had an estimated production of 509 tons of FW per year.
277 To estimate Tumaco's FW biogas potential the maximum biogas potential achieved in this study
278 (714.62 L biogas kg VS⁻¹, 62% CH₄) was used. Assuming that it is possible to collect the total
279 amount of FW produced (509 tons) it would be possible to generate 81,488 m³ of biogas/year,
280 equivalent to 489 MWh. This number was calculated by using an average biogas energy content
281 of 6 kWh m³ [37]. Likewise, assuming a biogas conversion efficiency to thermal and electrical
282 energy of 45% and 35%, respectively [38], up to 220 MWh of thermal energy or 171 MWh of
283 electric power could be obtained per year.

284

285 if all the biogas were purified and used to produce electricity, almost 230 homes could be supplied
286 with basic electricity consumption, which averages 62 kWh per month [39]. If all biogas production
287 were exclusively used for cooking, up to 230 families could supply their average basic
288 consumption of 18 m³ of propane gas per month (equivalent to 27 m³/month of biogas) [40]. If
289 biogas from FW were used for cooking each fishermen household will save around USD \$204 and
290 Tumaco's artisanal fishermen community would save around USD \$ 47.000 per year. This
291 calculation is based in the fact that families in Tumaco must buy propane or firewood for cooking
292 and it has an average price per month of USD \$ 17. As 42 kg of FW (89% VS) are necessary to
293 produce 27 m³ of biogas per month, a 6 m³ reactor (4.5 m³ work volume and 1.5 m³ for gas storage)
294 is required to maintain the optimum FW concentration (1% TS). A program for using FW to supply
295 the cooking energy demand of Tumaco's fishermen households can have an estimated cost
296 between 700 and 1.700 USD for household. This cost includes the installation of an 8-6 m³ plug
297 flow digester (Plug flow digesters do not require mechanical mixing and can be constructed by the
298 fisherman household for reducing costs) with a life span of 15 years with an estimated capital cost
299 between 500 and 1.500 USD [41]. The cost also includes the (~200 USD) artisanal fishermen
300 training for starting and maintaining the digesters (30 days HRT) and a 5 USD for water costs (0.2
301 USD per 1 m³ of water). The initial cost for this renewable energy and waste management program
302 in Tumaco have a payback period between 3 and 8 years for each household. The total cost of
303 this type of program for the National, state or city government is between 115.000 and 345.000
304 USD which is a low number compared with the cost associated with the negative effects produced
305 by mismanaging FW. Programs focused in changing the consumption of propane and firewood
306 for cooking will reduce the respiratory diseases caused by the combustion of firewood, the
307 production of greenhouse gases and the deforestation of mangroves around Tumaco.

308

309 **4 CONCLUSIONS**

310 The results of this investigation demonstrated the potential for using artisanal fish waste as a
311 substrate for methane production and provided a scientific and economic basis for developing
312 programs to utilise biomethane from FW to supply the energy demands of the artisanal fishermen
313 households in Tumaco, Colombia. Methane production from FW was possible at concentrations
314 up to 2% TS at mesophilic conditions (35-37 °C) with a Methane potential between 0.25 - 0.5 L
315 $\text{CH}_4 \text{ g VS}^{-1}$. The highest BMP was obtained at a FW concentration of 1% TS (464.5 mL $\text{CH}_4 \text{ g VS}^{-1}$),
316 higher concentrations had lower BMP as they were inhibited by the ammonia and VFA
317 accumulation. The treatments with 1.5, 2 and 2.5% TS exhibited diauxic growth as fats and
318 proteins had a different hydrolysis rate in the digester. It was estimated that Tumaco's annual FW
319 production (509 tons per year) can produce an energy potential of 489 MWh per year and can be
320 a solution for supplying the energy cooking demand of more than 200 artisanal fishermen
321 households. This research is the first step for establishing anaerobic digestion of FW as a solution
322 for managing Tumaco's FW and for improving artisanal fisherman health, and Tumaco's
323 environment and economy.

324

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333

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7 FIGURES AND TABLES LIST

Table 1. Physicochemical characterisation of FW and Inoculum.

Table 2. Economic analysis summary for Tumaco's artisanal FW biogas production.

Figure 1. Cumulative methane production and methane production rate for different FW concentrations. (a) 1% TS, (b) 1.5% TS, (c) 2% TS and (d) 2.5% TS. The values are means ± standard deviations (vertical bars, n = 3rd deviations).

Figure 2. Duncan grouping for means of FW concentrations ($\alpha=0.05$). Means covered by the same bar are not significantly different.

Figure 3. Ammonia production during anaerobic digestion of artisanal fish wastes at different TS concentrations.

Figure 4. Volatile Fatty Acids production during anaerobic digestion of artisanal fish wastes at different TS concentrations.

Table 1. Physicochemical characterisation of FW and Inoculum.

Parameter	Fish waste	Methanogenic inoculum
Moisture (%)	74,8	94,3
Total solids (TS) (%)	25,2	5,7
Volatile solids (VS) (%)	88,9	84,9
pH	7,4	7,7
COD (g/Kg) / (g/L)	265,0	93,59
C (%TS)	50,59	41,6
N (%TS)	8,84	8,9
C/N ratio	5,7	4,7
VFA (mg/Kg) / (mg/L)	1515,0	454,5
Ammonia (mg/Kg) / (mg/L)	627,2	487,2
Alkalinity (mgCaCO ₃ /Kg) / (mgCaCO ₃ /L)	650,0	1150,0

Table 2. Economic analysis summary for Tumaco's artisanal FW biogas production.

Tumaco's Artisanal fish landed per year	1131 tons
Tumaco's Artisanal FW per year (TAFWY)	509 tons
Potential biogas production from TAFWY	81,488 m ³ of biogas
Estimated energy production from TAFWY	489 MWh
Estimated thermal energy production from TAFWY	220 MWh
Estimated electric power from TAFWY	171 MWh

Figure 1. Cumulative methane production and methane production rate for different FW concentrations. (a) 1% TS and (d) 2.5% TS. The values are means \pm standard deviations (vertical bars, $n = 3$ rd deviations).

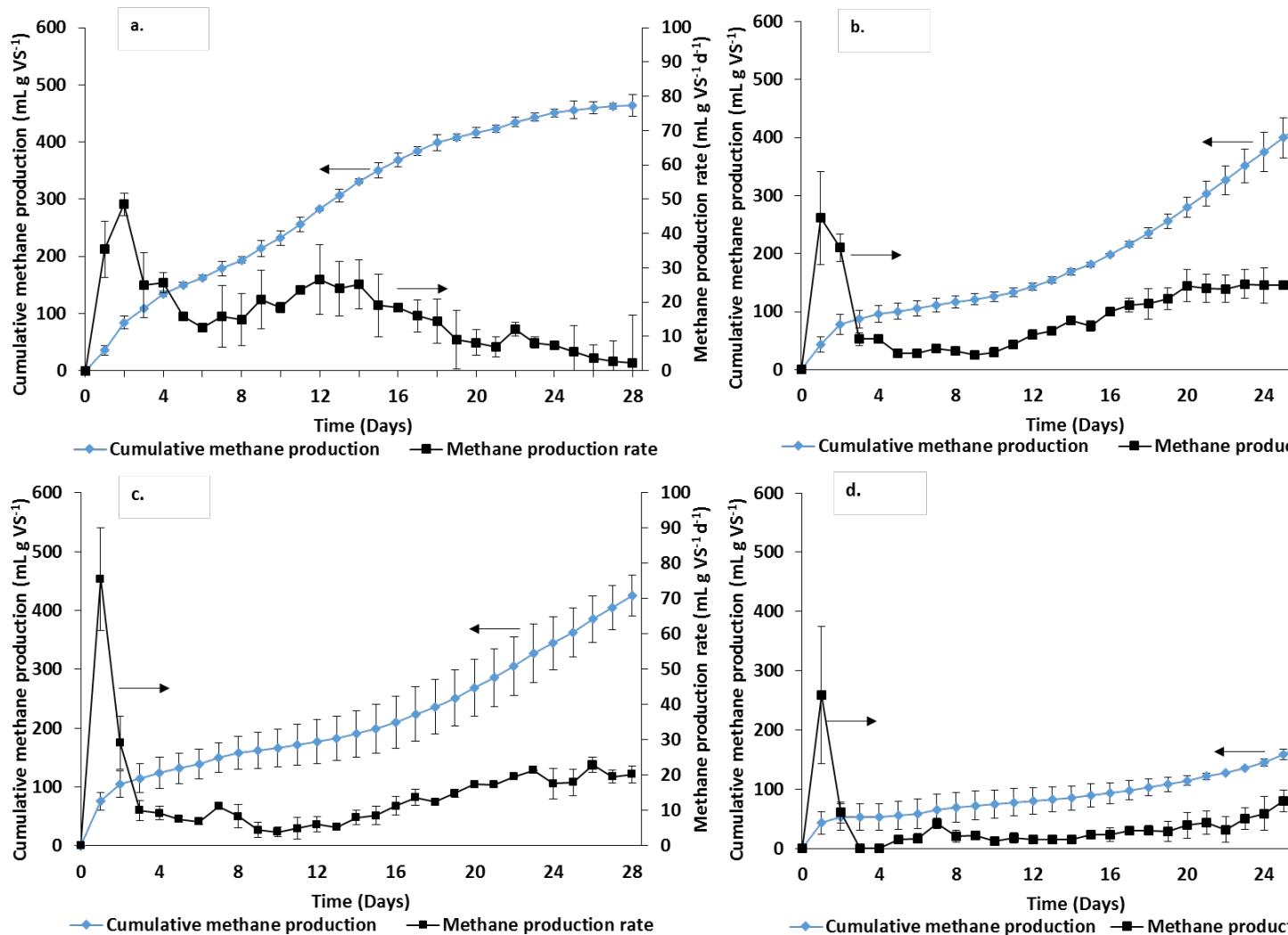


Figure 2. Duncan grouping for means of FW concentrations ($\alpha=0.05$). Means covered by the same bar are not significantly different.

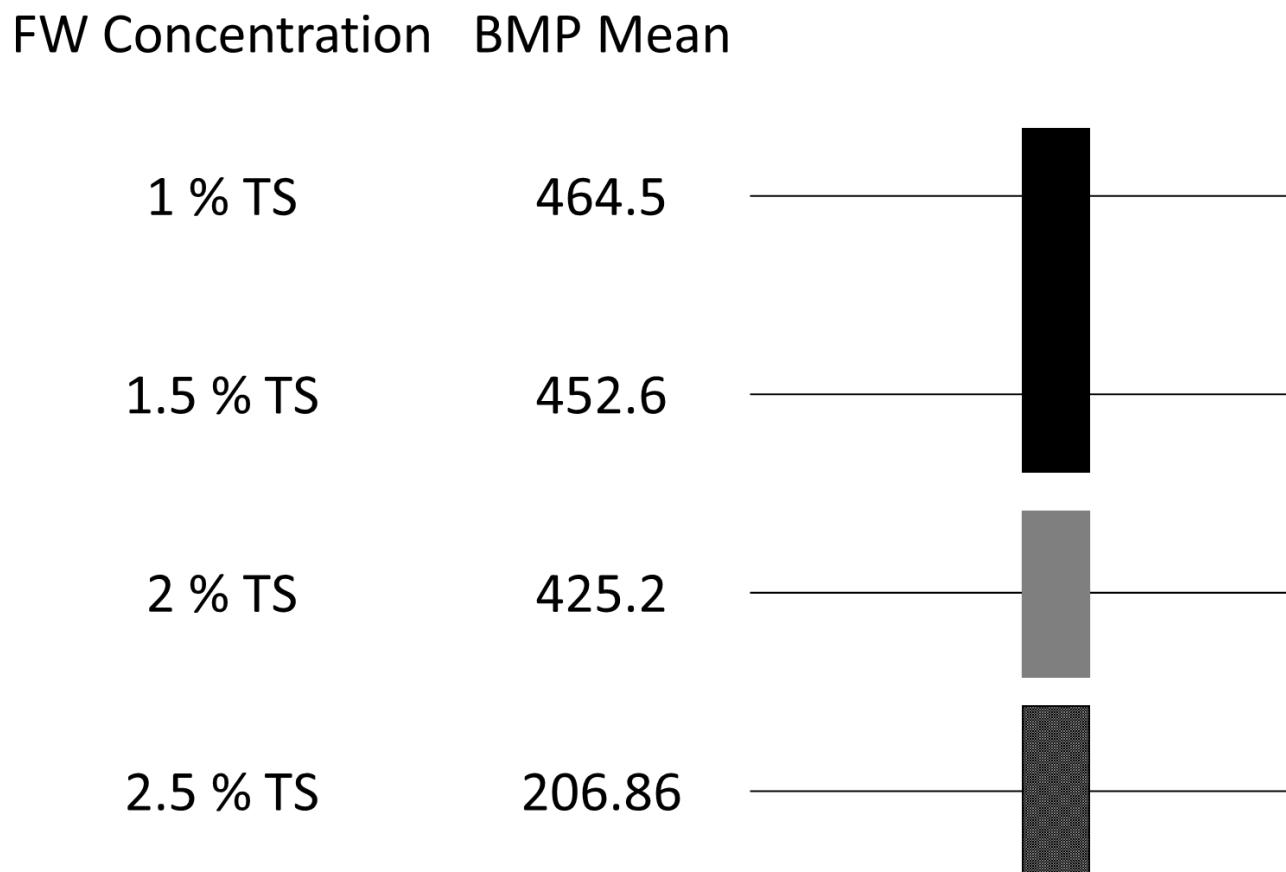


Figure 3. Ammonia production during anaerobic digestion of artisanal fish wastes at different TS concentrations.

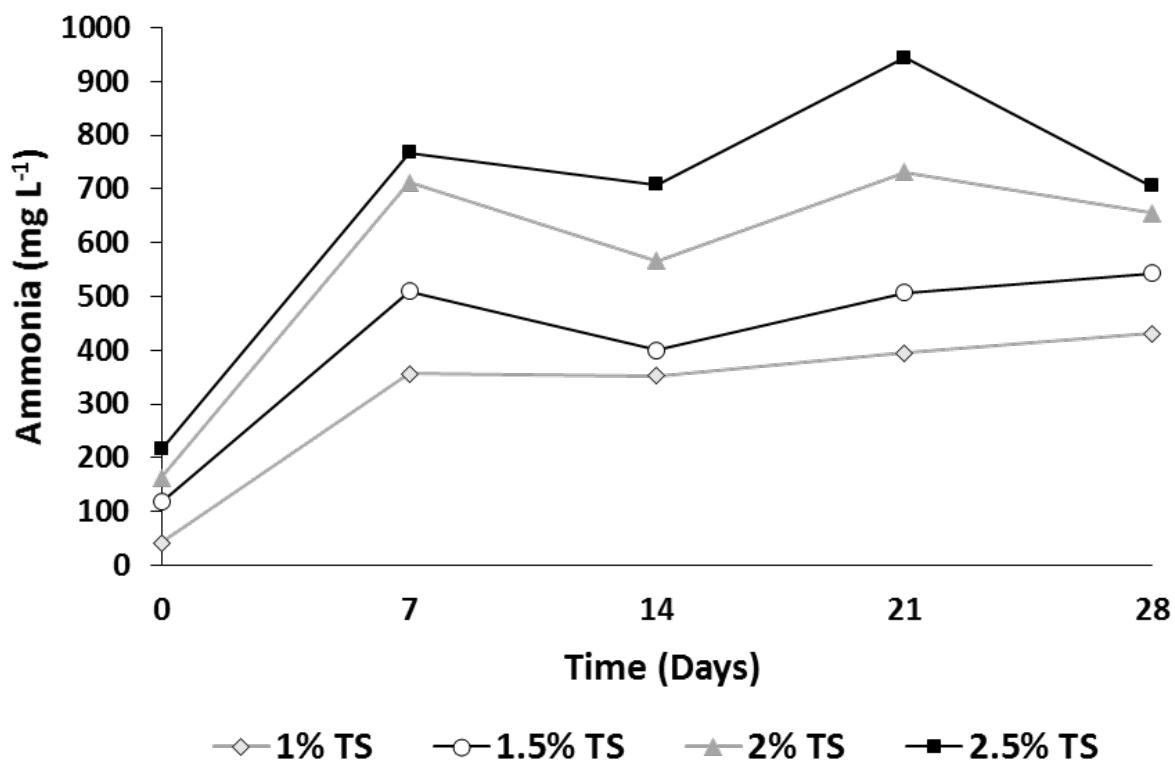
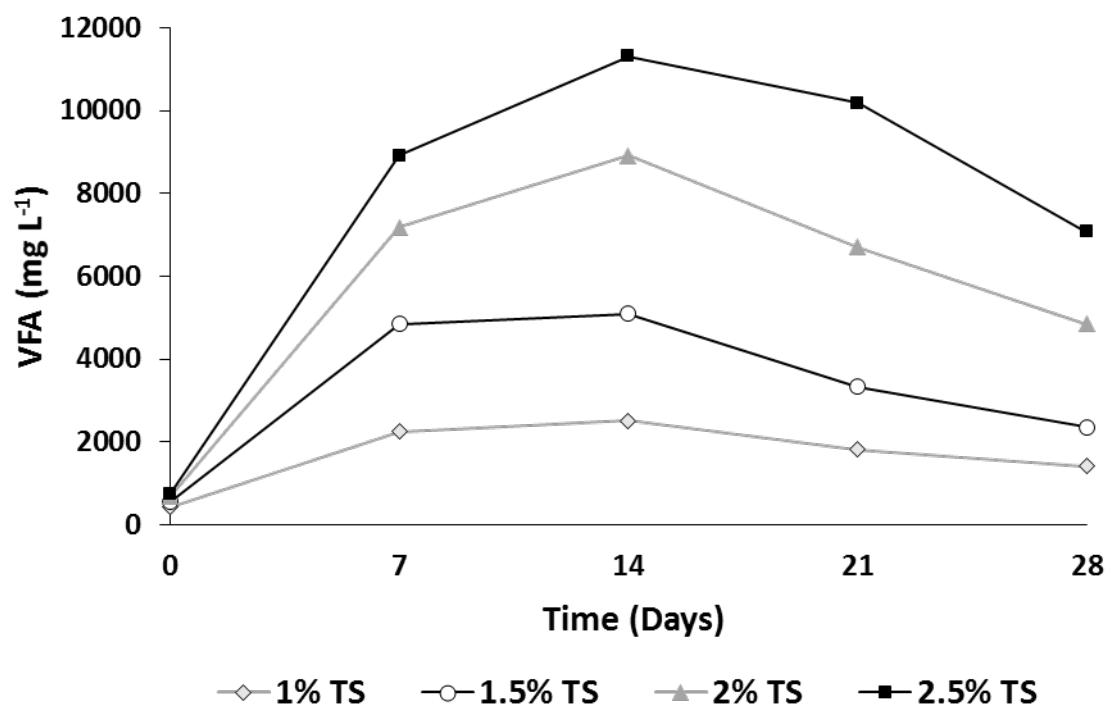


Figure 4. Volatile Fatty Acids production during anaerobic digestion of artisanal fish wastes at different TS concentrations



SUPPLEMENTARY MATERIAL

BIOMETHANE FROM FISH WASTE AS A SOURCE OF RENEWABLE ENERGY FOR ARTISANAL FISHING COMMUNITIES IN TUMACO, COLOMBIA

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Annex 1

Statistical Analyses

Class Level Information		
Class	Level s	Values
tratam	4	1%TS 1.5%TS 2%TS 2.5%TS

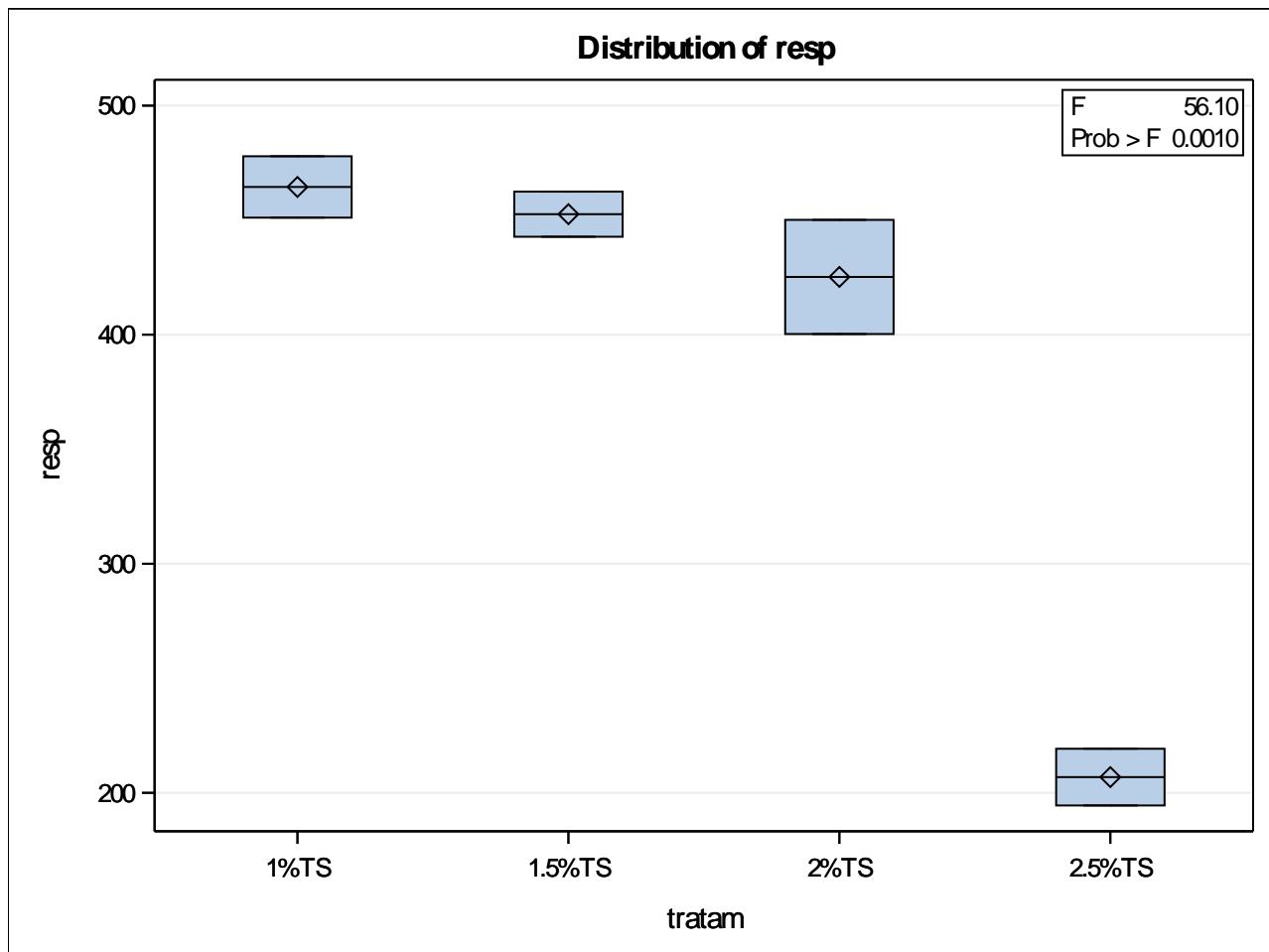
Number of Observations Read	8
Number of Observations Used	8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	88445.60414	29481.86805	56.10	0.0010
Error	4	2101.95425	525.48856		
Corrected Total	7	90547.55839			

R-Square	Coeff Var	Root MSE	resp Mean
0.976786	5.918902	22.92354	387.2938

Source	DF	Type I SS	Mean Square	F Value	Pr > F
tratam	3	88445.60414	29481.86805	56.10	0.0010

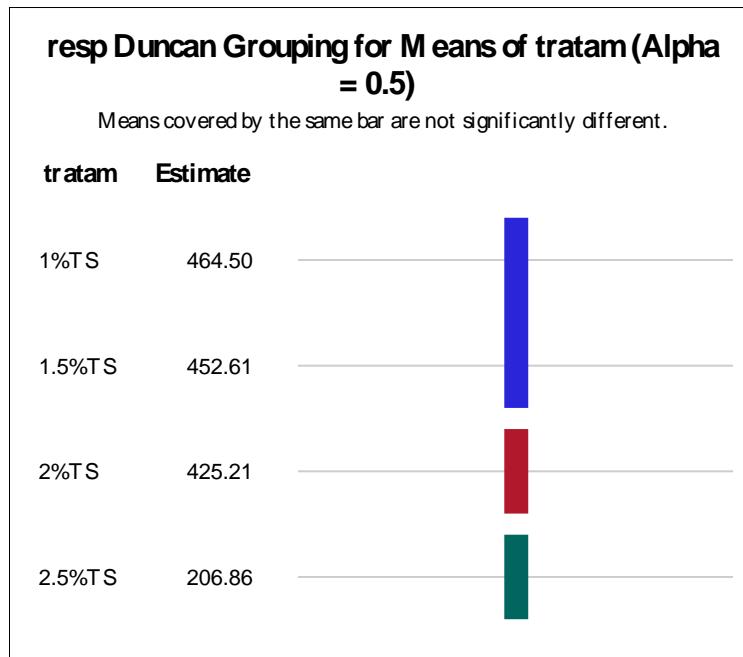
Source	DF	Type III SS	Mean Square	F Value	Pr > F
tratam	3	88445.60414	29481.86805	56.10	0.0010



Level of tratam	N	resp	
		Mean	Std Dev
1%TS	2	464.50000	18.9504617
1.5%TS	2	452.61000	13.9300036
2%TS	2	425.21000	35.2422020
2.5%TS	2	206.85500	17.5150350

Alpha	0.5
Error Degrees of Freedom	4
Error Mean Square	525.4886

Number of Means	2	3	4
Critical Range	16.98	17.19	17.06



Moments			
N	8	Sum Weights	8
Mean	0	Sum Observations	0
Std Deviation	17.3285654	Variance	300.279179
Skewness	0	Kurtosis	-1.4714732
Uncorrected SS	2101.95425	Corrected SS	2101.95425
Coeff Variation	.	Std Error Mean	6.12657305

Basic Statistical Measures			
Location		Variability	
Mean	0	Std Deviation	17.32857
Median	-284E-16	Variance	300.27918
Mode	.	Range	49.84000
		Interquartile Range	25.78500

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	0	Pr > t 	1.0000
Sign	M	0	Pr >= M 	1.0000
Signed Rank	S	0	Pr >= S 	1.0000

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.924541	Pr < W	0.4678
Kolmogorov-Smirnov	D	0.215127	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.070682	Pr > W-Sq	0.2435
Anderson-Darling	A-Sq	0.377664	Pr > A-Sq	>0.2500

Quantiles (Definition 5)	
Level	Quantile
100% Max	24.9200
99%	24.9200
95%	24.9200
90%	24.9200
75% Q3	12.8925
50% Median	-0.0000
25% Q1	-12.8925
10%	-24.9200
5%	-24.9200
1%	-24.9200
0% Min	-24.9200

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
-24.920	5	-9.850	4
-13.400	2	9.850	3
-12.385	7	12.385	8
-9.850	4	13.400	1
9.850	3	24.920	6