AdriAquaNet

Enhancing Innovation and Sustainability in Adriatic Aquaculture

Deliverable WP 3 - Activity 2.1

Waste management, emission reduction, renewable energy and energy saving

DL 3.2.1 Technical-scientific report on

THE POTENTIAL OF BIOGAS TECHNOLOGY APPLIED TO WASTEWATER FROM FISH FARMS, AND ASSESSMENT OF ENERGY RECOVERY AND ENVIRONMENTAL IMPACT REDUCTION

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CONTENTS OF THE DELIVERABLE

The present Document, constituting the Deliverable of WP 3 - Activity 2.1, is divided into 2 parts:

- **PART 1**

  The first part provides details and assessment of the WP objectives related to the primary subject of the report and the implementation and results, compared to the information already provided in the different progress reports, in order to give back a cumulative illustration of what the project delivered in relation to this task.

  This part is structured as follows:

  A. Report highlights
  B. WP 3 – Activity 2.1 output and results
  C. Durability and transferability of the project and its results
  D. Capitalization of the results
  E. Partnership cooperation
  F. Target groups involvement

- **PART 2**

  The second part provides the final results and a collection of data from the WP and project in relation to the General objectives at the Programme level that are also described in the final report of the Project.

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PART 1

A. REPORT HIGHLIGHTS

1) ***Context*** *– Aquaculture of the '20s has the capacity for further growth, but as it intensifies production, the sector must face the enormity of the environmental challenges, demanding new sustainable aquaculture development strategies (FAO, 2020).*
2) ***Challenge*** *– To develop and maintain high production levels and high-quality products of Adriatic aquaculture, while reducing negative effects on the environment and supporting energy demands with the production of alternative energies*
3) ***Expectations*** *– To contribute at reducing fish farm carbon footprint through Green-House Gas (GHG) emission reduction. To make fish hatcheries and farms more energetically sustainable.*
4) ***Solution*** *– To develop a wastewater treatment chain that recovers and uses fish waste for biogas production by anaerobic digestion (AD)*
5) ***We started by*** *establishing the most effective pre-treatments to obtain treatable sludges and testing if these sludges could be profitably treated by AD in marine and brackish conditions*
6) ***We then*** *determined at Lab-scale the biochemical methane potential (BMP) of hatchery brackish sludges, which showed a very high methane yield and therefore a promising opportunity to be exploited*
7) ***Finally,*** *we carried out several tests in pilot-scale anaerobic reactors, to establish the most effective process layout and the operational parameters of AD*
8) ***What we achieved*** *- a) the anaerobic process can be advantageously applied to fish farm effluents; b) fish farm effluents could be advantageously treated in co-digestion with other agricultural or mariculture waste or by-products; c) anaerobic technology could be applied at full scale, adopting the most effective operational parameters established through our experimental tests*
9) ***Our vision is to*** *improve the water quality by reducing pollution and the proportion of untreated wastewater while increasing recycling (Target 6.3 of the UN 2030 Agenda for Sustainable Development); to increase the share of renewable energy in the global energy mix (Target 7.2 of the UN 2030 Agenda for Sustainable Development); to achieve the environmentally sound management of all wastes (Target 12.4 of the UN 2030 Agenda for Sustainable Development); to reduce waste generation through prevention, reduction, recycling and reuse (Target 12.5 of the UN 2030 Agenda for Sustainable Development)*

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B. WP 3.2.1 OUTPUTS AND RESULTS

Increasing environmental sustainability of fish farming through fish farm waste management, emission reduction and renewable energy production by anaerobic digestion

B.1 INTRODUCTION

B.1.1 The State-of-the-Art

The production of fed finfish in marine or coastal aquaculture systems is mostly carried out with floating or submersible cages, which raises serious problems related to environmental impacts from many points of view. Seawater and sediment pollution, as a matter of fact, are the most serious and difficult environmental issues to be controlled, mainly due to the practical impossibility of collecting the released effluents from these sea systems (FAO, 2018; Price et al., 2015). The production of fish fries that are successively farmed in cages, instead, is normally carried out in land-based brackish and marine water systems, generally with tanks located in a protected environment. In order to reduce the overall water consumption, recirculation aquaculture systems (RAS) can be adopted. In RAS, the effluents separated from recirculating water can be collected and more carefully managed. Solid removal is an essential component in RAS, but often requires large quantities of water to wash out the accumulated waste (Timmons et al., 2007). Moreover, the produced waste is very diluted and for this reason it is difficult to store or submit to a proper treatment (Chiumenti et al., 2020).

The aerobic degradability of saline industrial wastewater was successfully demonstrated by comparing biological and chemical treatments in Kiel et al. (2014) and by using biological sequencing batch reactors (SBRs) in Uygur (2006), with a particular focus on nutrient abatement. However, considering that the aerobic processes are often characterized by high-energy demand and necessarily lack biogas production, in several cases the anaerobic digestion (AD) treatment is preferred, particularly when the concentrated effluents need to be treated (Figure 1).

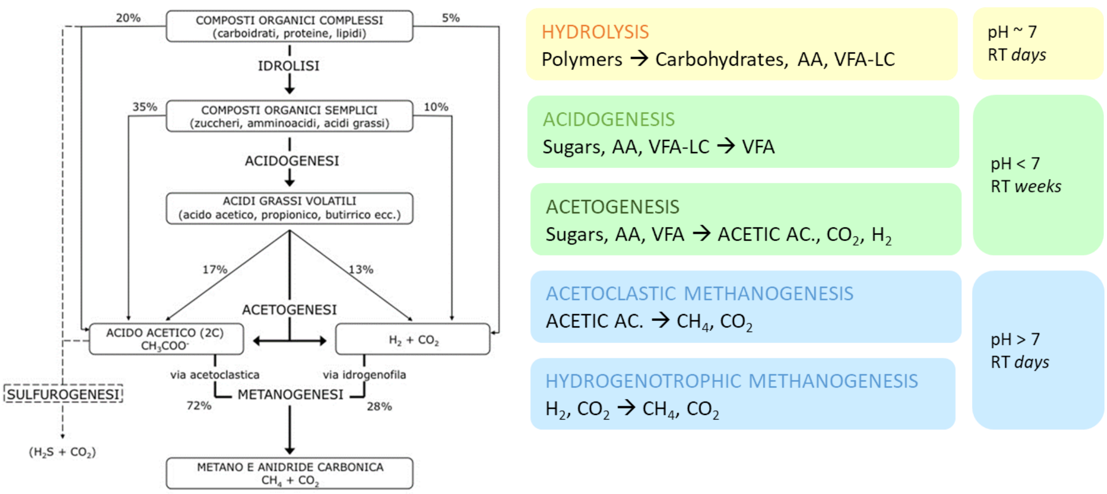

Figure 1. Schematic chemical pathways of anaerobic digestion. (Legenda: AA aminoacids, VFA-LC long chain volatile fatty acids, VFA volatile fatty acids, RT retention time).

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AD applied to marine or brackish aquaculture effluents could allow several advantages: the obtained biomethane can be used as an alternative source of renewable energy (Tal et al., 2009; Mirzoyan et al., 2010); the supernatant from AD may be recycled to RAS, saving water, salts, reducing energy costs and obtaining a lower final sludge volume (Mirzoyan et al., 2012); a reduction of the atmospheric pollution and carbon footprint can be accomplished (Chiumenti et al., 2009; Wongn et al., 2009); a reduction of sludge transport, disposal or reuse costs can be obtained (Reed et al, 1995).
However, some critical aspects can be associated with the anaerobic treatment of these sludges (Figure 2). AD is known to be inhibited by high salinity, mainly due to the presence of cations: in particular, a sodium concentration higher than 10 g/L strongly inhibits methanogenesis (Lefebvre et al., 2007). The anaerobic Archaea are able to adapt to moderate saline conditions, where methane production can be even stimulated, rather than inhibited (Misson et al., 2020). The high levels of sulphate, which is reduced to sulphide under anaerobic conditions, and ammonia produced by the degradation of protein in faeces and excess feed, can be other limiting factors in applying AD to aquaculture effluents (Gebauer, 2004).

Problem	Severity	Solutions
High dilution of waste: low concentration of dry and organic matter	TS <1% in the case study	Mechanical filtration / sedimentation and AD of thickened sludges (TS > 1%)
Salinity, Sodium concentration	Brackish water (10-17 g/l) in the case study	TEST of AD performances with brackish wastewater TEST of agricultural inoculum and saline adapted inoculum
High flows of wastewater	Flow rate 4-16 l/s in the case study	TEST of AD with not conventional reactors, biomass retention (UASB, UAF), semi-continuous reactors (AAS)
Competition with Sulphate reducing mo. (faster development than methanigens)	Described only in marine sediment, not in the case study	Use of agricultural inoculum, higher organic loading rates
Low biomethane yields	*To be verified by this Project*	Co-digestion process, Thermophilic regime, Not-conventional reactors

Figure 2. The anaerobic digestion of marine and brackish wastewater: problems and possible solutions.

In recent years, few studies have been addressed to the determination of the methane potential in AD from marine or brackish aquaculture effluents, mainly operating with lab-scale self-built apparatus; nonetheless, the reported results are particularly promising. For example, Gebauer (2004) treated marine aquaculture sludges with a continuously stirred tank reactor (CSTR), but the sludge was diluted 1:1 with tap water (5.3 g Na^+/L) to overcome inhibition and maintain a stable process, obtaining a methane production of 0.26–0.28 L CH_4/g of volatile solids (VS). Operating with an up-flow anaerobic sludge blanket (UASB) reactor and with a sludge salinity of 15–17 g/L, Tal et al. (2009) found a maximum specific methane production of 0.45 L CH_4/L of volume reactor/day. Zhang et al. (2014) employed 0.5 L batch reactors, reaching methane yields of 0.27–0.33 NmL CH_4/g VS in the treatment of sludge from the brackish fish farm; however, they used specific additives like glycine betaine and trehalose to increase the energy yield. Biochemical methane potential (BMP) assays allow establishing the methane production of liquid and solid substrates in the AD process through a standardized laboratory tool, giving useful information about substrate's biodegradability and methane generation kinetics. The BMP procedure involves adding small amounts of an active anaerobic inoculum and a selected substrate into serum bottles, creating anaerobic conditions and measuring gas production over time (Pearse et al,

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2018). The biochemical methane potential (BMP) of effluents from the farming of gilthead sea bream (*Sparus aurata*) was never determined before, according to the best of our knowledge. Consequently, the first specific aim of the present project was to find out if the AD could be technically applicable to treat sludges from brackish gilthead seabream farming. Moreover, it was fundamental to understand the optimal inoculum to substrate (I/S) ratio and the total methane potential of this substrate in order to have first indications on the feasibility of the AD process and on the eventual potential and requirements for a scale-up.

B.2 MATERIALS AND METHODS

B.2.1 Wastewater characterization and pre-treatments

The sludges used as a substrate for the determination of methane potential were collected from the fish hatchery located in Puglia (Ittica Caldoli, P9 of the Project), where fingerlings of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) are produced. Wastewater was discharged from the bottom of the tanks where fish were raised from 5 to 45 g l.w. and were filtered by a rotating drum filter (Hydrotech Microscreen 1603, Veolia) with a 30 mm mesh, having a treating capacity of 120 L/s (Figure 3). The thickened fraction from the filter was settled in a circular tank with a conic-shaped bottom and the sludges were collected after 1 h of sedimentation for the anaerobic tests (Figure 4). The inoculum was collected from a digestate of a full-scale mesophilic anaerobic digester (CSTR) treating dairy cow manure and corn silage. The main physicochemical characteristics of the actual substrate and inoculum are shown in Table 1. Before the beginning of the anaerobic assays, the inoculum was mixed and pre-incubated for a week at ambient temperature in order to volatilize the residual biogas and deplete the residual readily available organic material.

Figure 3. Layout of the recirculating aquaculture system (RAS) installed in PP9 fish farm.

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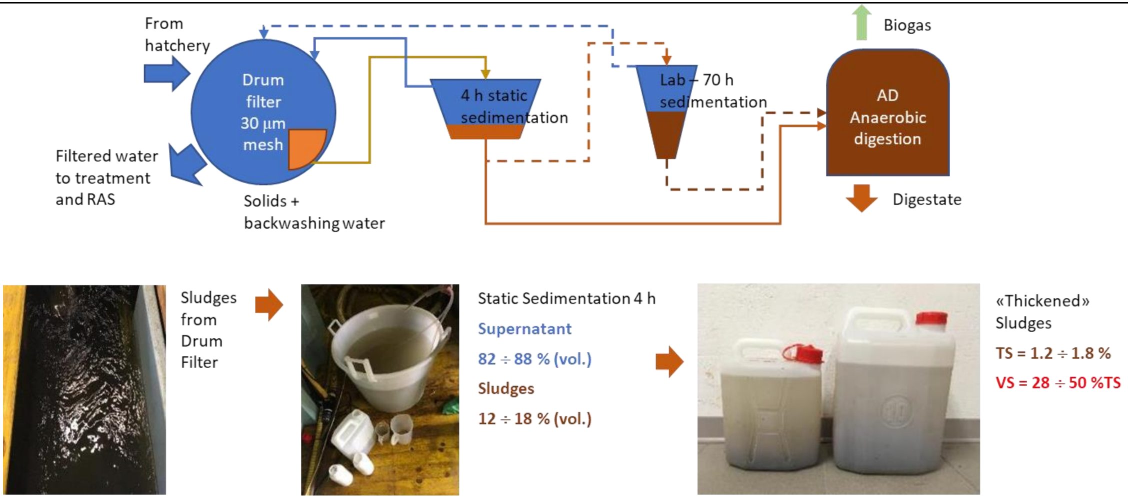

Figure 4. Pre-treatments applied to the wastewater for concentrating organic substances (thickened sludges). Legenda: RAS recirculating aquaculture system, TS total solids (dry matter), VS volatile solids (organic matter).

Table1. Physical-chemical characteristics of fish sludge and agricultural inoculum in the lab-scale tests (mean values and standard deviations).

Parameter	Units	Fish Sludge (Substrate)	Inoculum
Total solids (TS)	g/L	14.29 ± 0.3	64.27 ± 0.8
Volatile solids (VS)	g/L	4.70 ± 0.1	47.82 ± 0.6
Volatile solids (VS)	% TS	32.9 ± 1.5	74.4 ± 0.9
Total COD (tCOD)	mg/L	4045 ± 405	n.d.
Soluble COD (sCOD)	mg/L	3680 ± 220	8000 ± 75
Total Nitrogen (TN)	g/L	0.2 ± 0.07	4.53 ± 0.6
Ammoniacal Nitrogen (TAN)	g/L	0.011 ± 0.002	2.95 ± 0.5
Volatile fatty acids (VFA)	mg/L	596.5 ± 64.3	3115 ± 16.5
Total alkalinity (TAC)	mg/L	815.0 ± 0.1	17,058 ± 183
VFA/TAC	-	0.732	0.183
Salinity	g/L	12	-
Redox potential	mV	−60 ± 8	−328 ± 9
pH	-	5.9 ± 0.2	7.9 ± 0.0

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B.2.2. Lab-scale apparatus and batch tests (AMPTS system)

Biochemical methane potential (BMP) tests were carried out in an automatic methane potential test system (AMPTS, Bioprocess Control, Sweden). This equipment consisted of an incubation unit where 15 individual glass reactors were incubated at a constant temperature of 38° C. Each reactor had a total volume of 650 mL, and was filled with the mixture of inoculum and substrate for 400 mL before starting the tests. The reactors were mixed by an intermittent slow bent rotating agitator, operating for periods of 10 s every minute. The biogas produced in the reactors passed through an individual vial containing a 3 M NaOH solution, with the effect of retaining acidic gas fractions (mainly CO_2 and H_2S), allowing only CH_4 to flow through to the gas measuring unit. A solution of thymolphthalein as pH indicator was added to the NaOH vials for keeping the acid binding capacity of the solution. The volume of released CH_4 was measured using individual wet gas flow measuring devices, automatically recording the digital pulses at each filling of the flow cells (10 mL). A dedicated programmable logic controller (PLC) connected to a PC was used to elaborate and record the data. The cumulative methane production (expressed as NmL CH_4) and the net daily methane production of each reactor (NmL CH_4/day) were calculated by the difference between the CH_4 production from test bottles (substrate and inoculum) and the CH_4 production from blank reactors filled only with inoculum (standardizing the VS content). The final BMP value was then expressed as the cumulative methane yield at the end of the test divided by the relative VS content (NmL CH_4/g VS), as recommended by Pearse et al., 2018. On the basis of BMP tests previously performed with diluted effluents (Mainardis et al., 2019), a wide range of I/S ratios, based on the relative VS content of inoculum and substrate, were tested in this preliminary campaign, including the following values: I/S = 50 (IS50); I/S = 30 (IS30); I/S = 3 (IS03). In addition, the substrate itself, without inoculum, (S100) was tested to evaluate its eventual methane production in absence of a specialized anaerobic microorganisms consortium. The tests were carried out in triplicate, and 3 reactors filled only with inoculum were used as blanks to calculate the net methane production. All the experimental data were statistically analysed using one-way analysis of variance (ANOVA) through the MS Excel software, at a level of significance $p < 0.01$.

B.2.3. Pilot scale apparatus and continuous loading tests (BRS system)

The Bioreactor Simulator System-BRS (Bioprocess Control, Sweeden), acquired in the frame of the Project, is a pilot scale plant capable of semi-continuous or continuous operations, simulating a full-scale AD plant. The system can be observed in Figure 5, and is composed of 6 glass bioreactors of 2.0 L volume, manually loaded with the input effluent. The digesters are equipped with a mechanical mixing system, operating at pre-set intervals, speed and direction (maximum 200 rpm). Temperature control is achieved by a thermostatic bath set at 38.0 °C. Each digester is equipped with an individual gas line, composed of a pipe that conveys the biogas to a purification unit, containing an alkali solution that removes the acidic fraction (mainly H_2S e CO_2) from the biogas, and finally the methane is conveyed to a flow-cell measurement unit (resolution 10 mL). In the start-up phase, the digesters were filled with the different inocula (B2 generic, B1 specific brackish, and M1 adapted marine) and were maintained in batch conditions for 20 days. Subsequently the progressive load of fish effluents started, divided in 5 different sequential phases for a total of 117 days. Three theses were compared (Table 2): for M1 and B1 equal organic loading rates (OLR) and hydraulic retention times (HRT) were kept, but the first was maintained in marine salinity conditions while the second was maintained brackish; for B2, instead, lower OLRs and higher HRTs were kept in phases 3-5 (Table 2). In detail, the HRT was reduced from the initial value of 30.0 days to 12.9 days for M1 and B1, and from 30 to 18.9 for B2, with OLR increased from 0.16 g VS/L day to 0.64 g VS/L day and to 0.43 g VS/L day for M1-B1 and B2 respectively. Initially the loading was

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performed twice per week (phases 1-2), and the frequency was later increased to 3 loads per week (phases 3-4) and to 5 loads per week (phase 5).

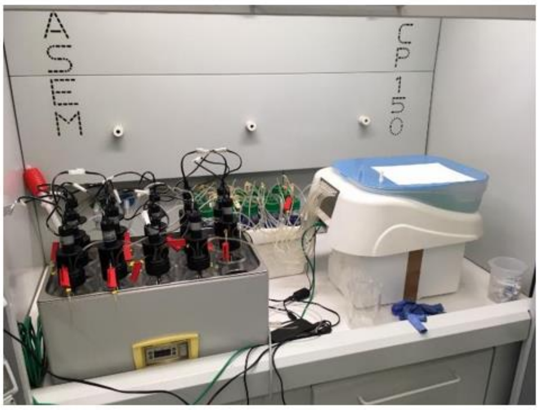

Figure 5. The AMPTS automatic methane potential test system (Lab-scale, on the left) and the BRS bioreactor simulator system (the Pilot-scale system, on the right). The BRS is acquired in the frame of the Adriaquanet Project.

Table 2. Physical-chemical characteristics of fish sludge and inoculum in the pilot-scale tests (mean values and standard deviations).

Parameter	Inoculum B2	Inoculum B1 and M1[a]	Substrate P 1 	Substrate P 2-5 
TS (g/L)	64.27 ± 0.8	42.23 ± 4.53	14.08 ± 0.30	17.65 ± 0.69
VS (g/L)	47.82 ± 0.6	16.05 ± 1.72	4.79 ± 0.12	8.97 ± 1.24
VS/TS (%)	74.4 ± 0.9	38.0 ± 0.1	34.0 ± 1.5	49.5 ± 4.2
COD_t (mg/L)	n.d.	n.d.	4,045 ± 405	9,868 ± 653
COD_s (mg/L)	8,000 ± 75	14,350 ± 525	3,680 ± 220	8,980 ± 594
TKN (g/L)	4.53 ± 0.60	2.68 ± 1.32	0,30 ± 0,16	0,37 ± 0.01
TAN (g/L)	2.95 ± 0.50	2.27 ± 0.99	0.01 ± 0.00	0,02 ± 0.00
VFA (mg/L)	3,115 ± 16.5	1,961 ± 996.0	597 ± 64.3	794 ± 30.9
TAC (mg/L)	17,058 ± 183	12,745 ± 738.5	815 ± 0.0	1,085 ± 42.2
VFA/TAC	0.183	0.161	0.732	0.730
Salinity (g/L)	0.0	11.5 ± 1.5 / 35.0[a]	12.0	12.0
Redox p. (mV)	-328 ± 9	-376 ± 10	-60 ± 8	-250 ± 11
pH	7.90 ± 0.0	7.63 ± 0.23	5.9 ± 0.2	5.9 ± 0.2

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B.2.4. Study and optimization of a new type of reactor

The BRS system consists of 6 reactors with a usable volume of 1.8 L each: 3 reactors were used in conventional operating conditions (CSTR, continuous stirred tank reactor), while 3 reactors were modified with an up-flow fixed bed layout, with floating plastic filling elements (UAFF type, Figure 6).

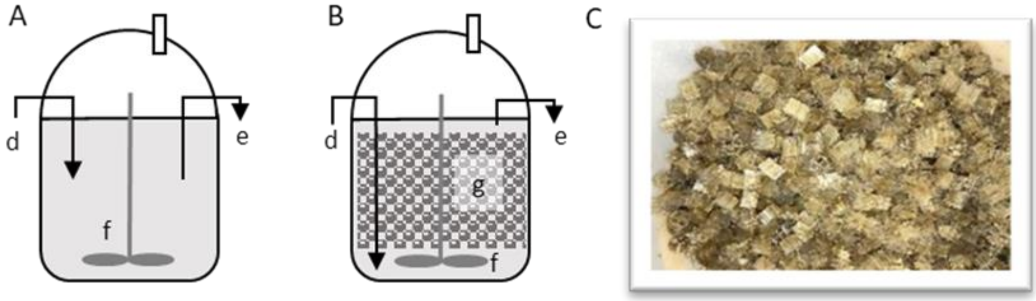

Figure 6. Continuously stirred tank reactor (CSTR, A), upflow anaerobic floating filter (UAFF, B) and detail of the plastic filling materials colonized by anaerobic biofilm. In evidence: loading line (d), discharge (e), mechanical stirrer (f), floating filter (g).

The experimentation was carried out in two phases (Table 3): the first phase, "BRS colonization", lasted for a total of 72 days and was characterized by the use sludge from a settling tank of orate wastewater as substrate, and of digestate derived from previous tests of anaerobic digestion of brackish hatchery effluents as inoculum. The UAFF reactors were equipped with media previously colonized with the same digestate used as inoculum.

The second phase "BRS Anaerobic tests" was performed with a semi-continuous load of filtered effluent from the RAS system of the hatchery. The load was performed on a daily basis. Digestate originated in the first batch phase was kept in the reactors, and the hydraulic volume was progressively replaced by the effluent from RAS. A preliminary step called "Start" was characterized by the first complete hydraulic replacement of reactors, obtained in 6 days. The test lasted for a total of 114 days. The feeding rate of reactors was adjusted in order to achieve hydraulic retention times (HRT) of 20, 12 and 8 days that characterized 3 following steps (step 1, 2 and 3).

B.3 RESULTS

B.3.1. The Biochemical Methane Potential (BMP) of Fish Sludges

The broad range of tested inoculum to substrate ratios produced significantly different cumulated methane productions (Figure 7). The highest production was obtained from the highest tested ratio (IS50), while the reduction of relative inoculum proportions evidenced gradually lower productions. In more detail, looking at methane production curves, IS50 and IS30 performed very similarly until day 20, after which IS30 ceased to increase, while IS50 continued to grow up, and slowed down only after day 26. The production trend of IS03 was different from the previous ones, as it increased more rapidly until day 10, but then it grew slowly until it stabilized on lower final BMP values. As could be expected, the growth of S100 (only substrate) methane production was very slow and the methane production trend was much more flat than the other curves.

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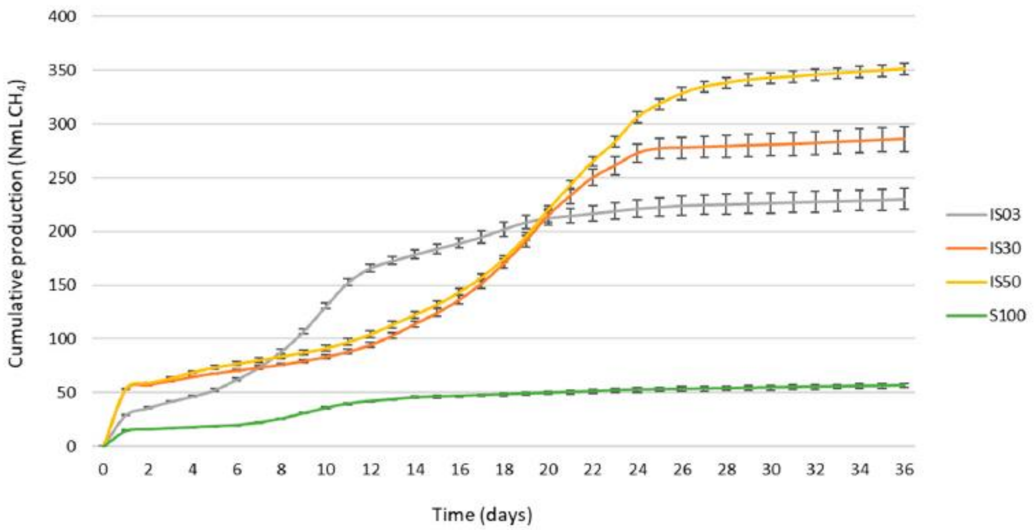

Figure 7. Cumulative methane production during lab-scale BMP tests. Legenda: BMP biochemical methane potential, IS inoculum to substrate ratio.

The trends of daily methane productions depicted in Figure 8 allow to better highlight these differences among the experimental theses. The daily production of IS03 showed the highest peak on day 10 (23.4 NmL CH_4/day), while for IS30 and IS50 the peaks occurred almost simultaneously around day 20–21, however with values that were not very different from those of IS03 (25.1 and 23.7 NmL CH_4/day, respectively for IS50 and IS30). The daily production of S100 reached the maximum on Day 9 (as for IS03), but with lower NmL CH_4/day). Moreover, less pronounced peaks of daily methane production were observed for all the theses, probably due to the degradation of less easily digestible compounds, at day 14, 18, 25 and 26, respectively for S100, IS03, IS30 and IS50 trials. Similar behaviours are known and well described for other substrates, such as for example cheese whey (Mainardis et al., 2019), paper sludge (Priadi et al., 2014), potato peels (Liang et al., 2015; Achinas et al., 2019). The Biochemical methane potentials obtained with different I/ S ratios differed significantly, with values gradually decreasing from the highest inoculum to substrate ratio (IS50) to the total absence of inoculum (S100).

BMP mean values are reported in Figure 9. The highest obtained BMP (564.2 ± 16.9 NmL CH_4/g VS, in IS50) was comparable to the yields of 396.8—779.8 NmL CH_4/g VS obtained in previous tests on thickened sludge from rainbow trout farming in freshwater (Chiumenti et al., 2020) and to the highest yield of 460 L/ kg VS reported for effluents derived from rainbow trout farming in freshwater (Lanari et al., 1998). Moreover, the highest BMP yield in this study resulted higher than a previous literature outcome of 270—330 NmL CH_4/g VS, reported with sludges of turbot (*Scophthalmus maximus*) farmed in brackish water with 15.2 g/L salinity in an AMPTS system (Zhang et al., 2014). However, additives like glycine betaine and trehalose were used in that research to improve the biogas production rates, while in the present study no additives were used. Other research studies have been carried out with different laboratory methods, which have not always allowed to reach the determination of BMP value but gave useful indications for a comparison with the present study. For example, methane yields of 0.45 L CH_4/kg input sludge have been determined from gilthead sea bream farming in brackish conditions and with a continuous load AD process (Mirzoyan et al., 2013). Gebauer (2004) obtained methane yields of 160—241 NmL CH_4/ g VS from Atlantic salmon farming, reducing the saline content from 35 to 17.5 g/ L

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respectively. The inoculum used in these studies was municipal sewage sludge and cow manure (Gebauer, 2004) or digestates from AD of various fish waste adapted to saline conditions (Zhang et al., 2016; Gebauer et al., 2006; Mirzoyan et al., 2013). The present experimentation demonstrated good methane yields, even without the use of a specialized inoculum or a community already adapted to saline conditions, and provided that a sufficiently high inoculum/substrate ratio is maintained.

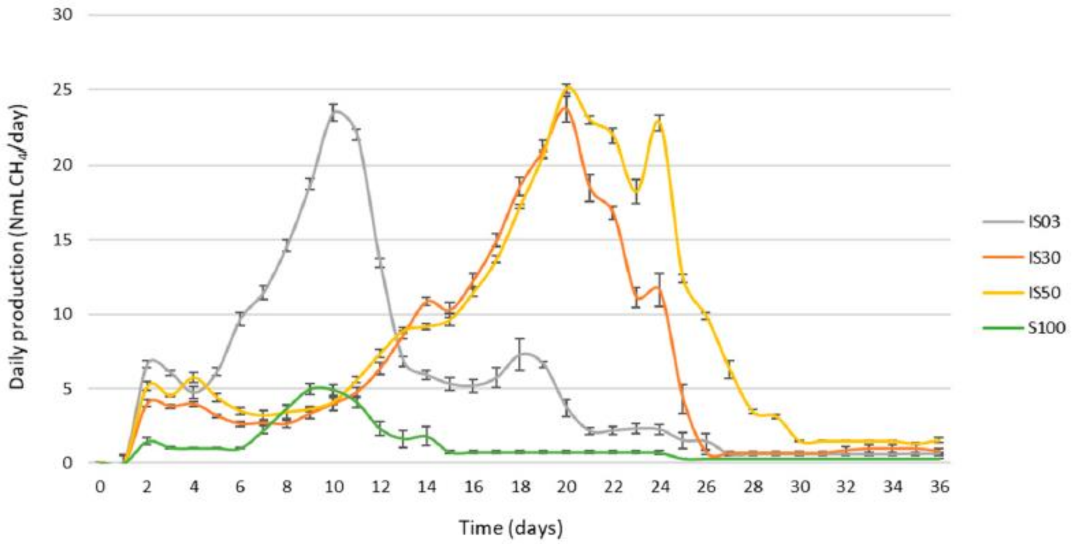

Figure 8. Daily methane production during lab-scale BMP tests.

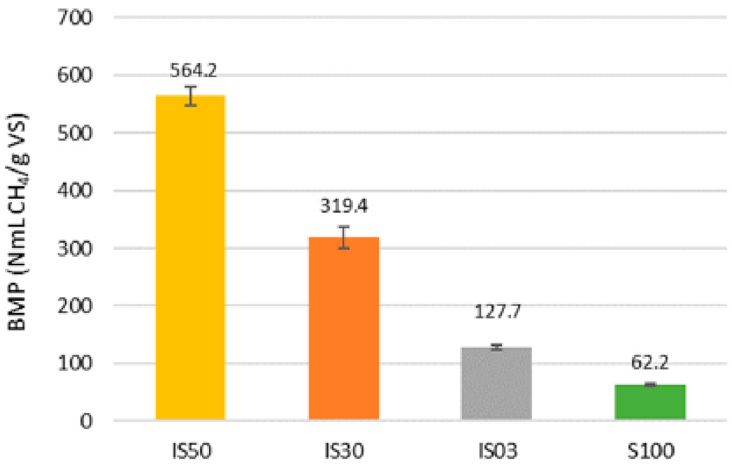

Figure 9. Biochemical potential values (BMP) of fish sludges. Legenda: IS inoculum to substrate ratio.

B.3.2. Optimization of process parameters at pilot scale

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During the adaptation phase (days 0-20), the non-specific generic inoculum showed higher production of methane than the other inocula, but at the end of the period the daily production resulted stable or in slight decrease for all. In the following loading phases (phase 1, up to the 55th day), the progressive substitution of the useful volume of the digesters took place and M1 started showing lower production (104.1 NmL/g VS) than B1 and B2 with similar performances (186.4 - 187.9 NmL/g VS), equal OLR but different starting inocula. In phase 1, the generic inoculum (B2) showed a similar methane potential than the specific inoculum, leading to the supposition that methanogenic Archaea could be capable of a rapid adaptation to a moderately saline environment (Misson et al., 2020; da Borso et al., 2021). In order to operate in marine conditions, a multistep adaptation process with an initial increase of salinity to critical conditions followed by a further slight and progressive increase could reduce the start-up period without negative consequences on methane production (Kimata-Kino et al., 2011).

During all phases, M1 showed lower performance than B1 and B2, but showed an evident improvement with the increase of the OLR, reaching the maximum yield (241.2 NmL/g VS) with OLR of 0.38 g VS/L day, only slightly lower than B1 (256.3 NmL/g VS) at the same loading rate. B1 e B2 showed a similar trend, but with a higher yield for B2. In particular, going from P2 to P3, B1 showed an increased yield from 237.4 to 256.3 NmL/g VS while B2 went from 245.9 to 264.0 NmL/g VS.

Methane yields of all the digesters decreased during phase 5, performed with higher OLR (0.64 e 0.43 g VS/L.day, for M1-B1 e B2 respectively) and 5 loads/week, resulting in a CH_4 production of 186.3, 185.4 and 156.7 NmL/g VS respectively for B2, B1 e M1. The loading modality clearly affects the stability and the efficiency of the AD process (Pramanik et al., 2019).

The results lead to consider favourable to maintain a maximum OLR of 0.42 g VS/L in brackish conditions and not less than 20 days of HRT, in order to limit the biomass washout from the digesters. This combination of parameters is in contradiction with existing literature that considers longer HRTs and lower OLRs to limit the accumulation of volatile fatty acids with maximum and constant yield (Pramanik et al., 2019).

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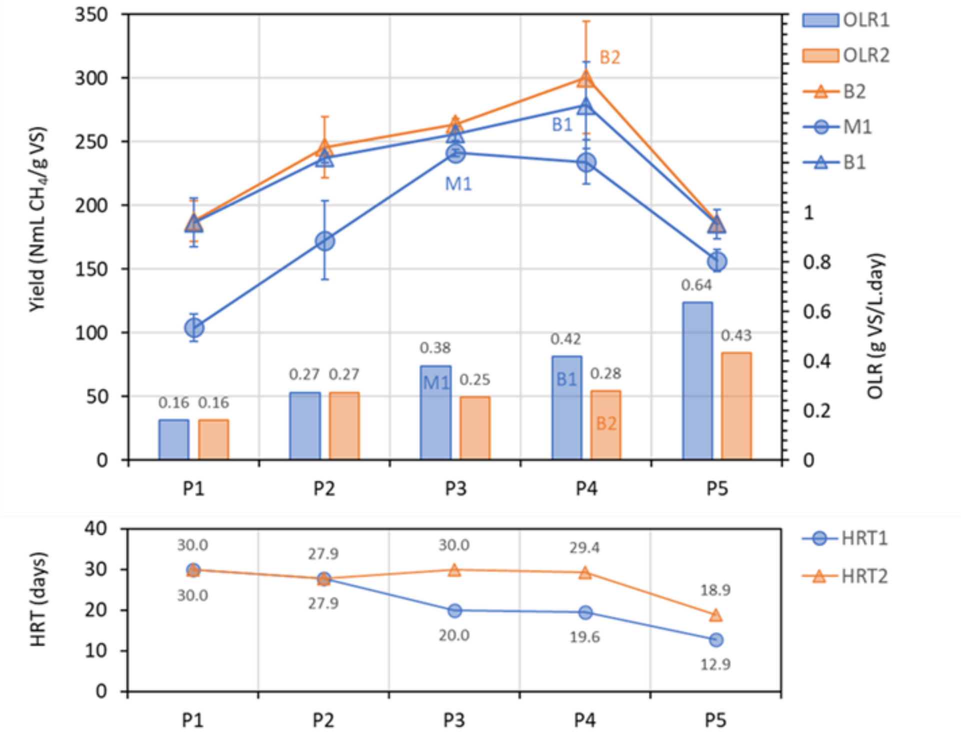

Figure 10. Methane yields obtained during the pilot-scale tests of fish sludges in marine (circles) or brackish (triangles) conditions, adopting different retention times and corresponding different organic loading rates (blue and orange colours). Legenda: OLR organic loading rate, HRT hydraulic retention time, M1 marine at HRT 1, B1 brackish at HRT 1, B2 brackish at HRT 2.

A possible further development of the present research could be the fractioning of the load into smaller doses to be fed at least on daily basis, considering that a regular loading results in a higher and more constant productivity (Gebauer & Eikebrokk, 2006).

The lower methane yields compared to freshwater effluents (Chiumenti et al., 2020) could depend on the salinity level. A scientific study on the AD of saline wastewater, with increased acidity, showed that methanogenic activity is present even at high salinity levels (30.0 g/L) but underlining also an extremely small particle size with possible risk of losing the microbial biomass in the discharge operations (Jeison et al., 2008). With AnMBRs (Anaerobic Membrane Bioreactors) it was possible to retain in the digester small size active particles, allowing to increase the OLR and subsequently the methane production (Jeison et al., 2008). In another research, an hybrid FBR (Fixed Bed Reactor), consisting of a fixed bed up-flow anaerobic sludge blanket (UASB) reactor, was tested for the treatment of winery wastewater: the adoption of media for biomass retention, represented by small porous polyethylene cylinders, allowed to increase the OLR, and hence the treatable wastewater, without affecting the COD removal efficiency (Ganesh et al., 2010). A recent experimentation about the digestion of rainbow trout (*Oncorhynchus mykiss* Walbaum) fresh wastewater showed that the HRT could be lowered maintaining an optimal methane yield (486.7 NmL CH_4/g VS), by operating with an hybrid fixed bed UASB reactor, with possible reduction of the digestion volume in case of a scale up of the process, with lower investment and operational costs (Chiumenti et al., 2020).

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B.3.3. Study and optimization of the new type UAFF reactor

The substrate used to colonize the CSTR and UAFF type reactors during phase 1 was characterized by brackish conditions (salinity 13‰), with an average pH of 7.7, TS of 4.754 % and VS of 52.6% referred to TS (Table 3).

Table 3. Operative parameters and physical-chemical characteristics of the inputs during the pilot-scale experiments testing new type of UAFF reactor (upflow anaerobic floating filter reactor).

Parameter	Phase 1	Phase 2			
	Colonization	Start	Step 1	Step 2	Step 3
Operational Parameters					
Loading mode	Batch	Daily	Daily	Daily	Twice/Day
Hydraulic Retention Time, HRT (days)	Discontinuous (72 days)	15	20	12	8
Organic Loading Rate (g VS/L.day)	37.1 ± 3.5*	0.212	0.159	0.264	0.396
Chemical characteristics					
Salinity (‰)	13 ± 0.6	13 ± 0.6			
pH	7.77 ± 0.13	6.59 ± 0.21			
Total Solids, TS (%)	4.754 ± 0.268	1.269 ± 0.02			
Volatile Solids, VS (%)	2.493 ± 0.048	0.338 ± 0.02			
VS/TS (% TS)	52.6 ± 3.2	26.6 ± 1.3			

The colonization test phase 1 lasted 72 days, during which the reactors inoculated with filing media (UAFF) showed higher methane production in comparison with the control units (CSTR) (Figure 11). The final yield resulted significantly superior for the UAFF, with values of 187.9 ± 15.1 Nml CH_4/g VS (referred to input VS) in comparison with 100.0 ± 16.7 Nml CH_4/g VS of the CSTR (Figure 12). The daily production of methane rapidly increased in the first week of the treatment, reaching the peak values of 15.0 e 8.1 Nml/g VS/day for the UAFF and CSTR respectively between the 3rd and 4th day (Figure 13). Subsequently, the daily production progressively decreased, reaching values below 1 Nml CH_4/g VS/day for both lines from the 39th day.

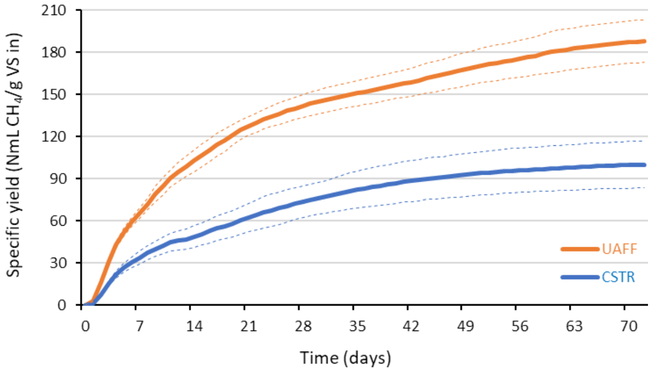

Figure 11. Cumulated specific yield observed during phase 1. Legenda: CSTR conventional continuously stirred tank reactor, UAFF new type upflow anaerobic floating filter reactor.

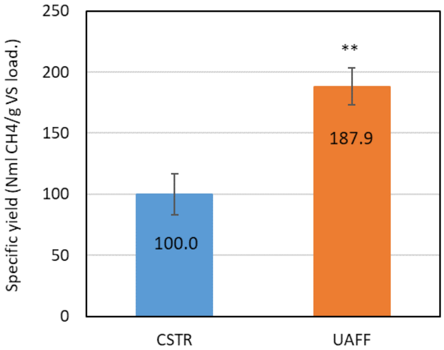

*Figure 12. Methane specific yield for the CSTR and UAFF reactors. (** P < 0.01).*

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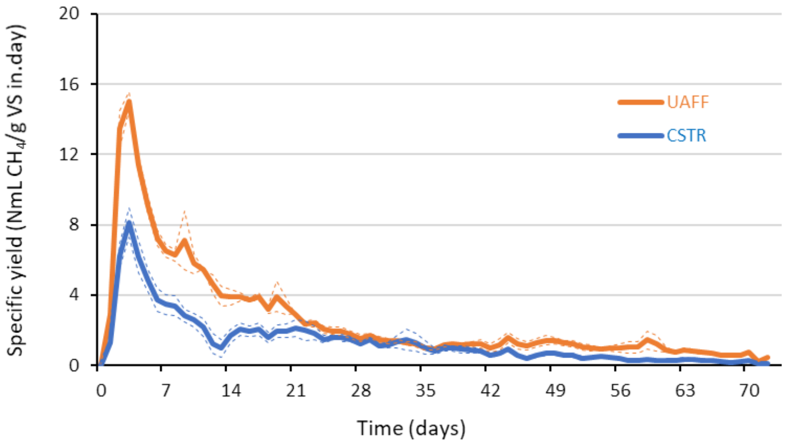

Figure 13. Evolution of the specific methane yield, on daily basis, recorded in phase 1.

During phase 2, the input substrate was loaded daily (Start, and Steps 1 and 2) or twice a day (Step 3). The input presented relevant dilution, with average TS content of 1.269 ± 0.023 %, and low VS content, in detail 0.338 ± 0.023 % (see previous Table 3), despite the pretreatment by mechanical filtration and the collection of the thickened fraction after 4 hours of settling. Furthermore, the substrate presented brackish salinity conditions (13 ‰) and pH of 6.59 ± 0.21.
With a volumetric load of 0.12 L/day, corresponding to a specific organic load of 0.212 g VS/L day, the complete replacement of the volume of reactors took place in 15 days, a period considered as the end of Phase 2-Start (Table 3). The three following steps were characterized by the progressive reduction of the HRT from 20 to 12, and then to 8 days (for Step 1, Step 2 e Step 3 respectively), and hence operating with OLRs of 0.159, 0.264 e 0.396 g VS/L day, for the three steps respectively (Table 3).
The cumulated volumetric production of methane recorded for the entire duration of phase 2 presented an increasing trend as the effect of the increasing organic load (Figure 14), and UAFF reactors generally showed higher performance than the CSTR. The evolution resulted almost linear in the different steps, but with an increase of the slope during Steps 2 and 3, both characterized by increasing OLRs. Furthermore, UAFF production increased progressively and more evidently than CSTR production.

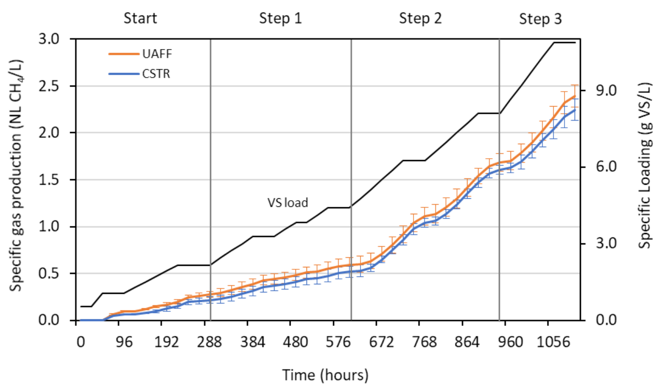

Figure 14. Cumulated volumetric production of UAFF and CSTR digesters, and specific organic load adopted in Phase 2, and different steps with increasing HRT. The bars represent the standard deviations.

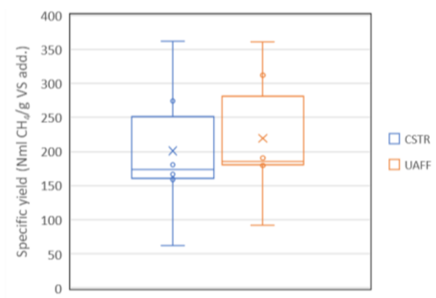

Figure 15. Plot of the specific yields in Phase 2. The X point represents the average value, the horizontal line is the median.

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Considering the entire data set from Phase 2, a box-and-whiskers-plot was drawn (Figure 15): the graphics clearly highlight the higher yields obtained by the UAFF compared to the CSTR, despite showing comparable values higher than 350 Nml CH_4/g VS add. For both lines of digesters. The average specific yields resulted in 219.5 Nml/g VS for UAFF and 200.9 Nml/g VS for CSTR, but with a wide variability of data evinced in consequence of the different operational parameters, as expected.
The detailed analysis of the specific yields recorded in each experimental step (Figure 16) allowed to draw interesting observations, and in some cases significant results. As expected, the Start Phase showed lower productions of methane in comparison to the other phases: in fact, in this phase the digestate from Phase 1 was gradually replaced by the daily load of fresh substrate. The start-up of the process seemed more rapid in the UAFF digesters, in comparison with the CSTR, with a specific yield of 92.5 Nml CH_4/g VS that resulted significantly higher than 62.5 Nml/g VS recorded for the CSTR. In Step 2, and more evidently in Step 3, the specific yield showed a relevant increase, reaching values of 180.9 – 190.5 Nml CH_4/g VS and 361.3 – 360.5 Nml CH_4/g VS (for CSTR and UAFF, in Step 1 and Step 2 respectively). With a further reduction of the HRT to 8 days, despite a consequent increase of the OLR to 0.396 g VS/L day, the yields resulted decreased in comparison with those obtained with an HRT of 12 days, but the specific yield recorded for the UAFF, equal to 312.0 Nml CH_4/g VS, resulted significantly higher than CSTR (274.5 Nml/g VS).

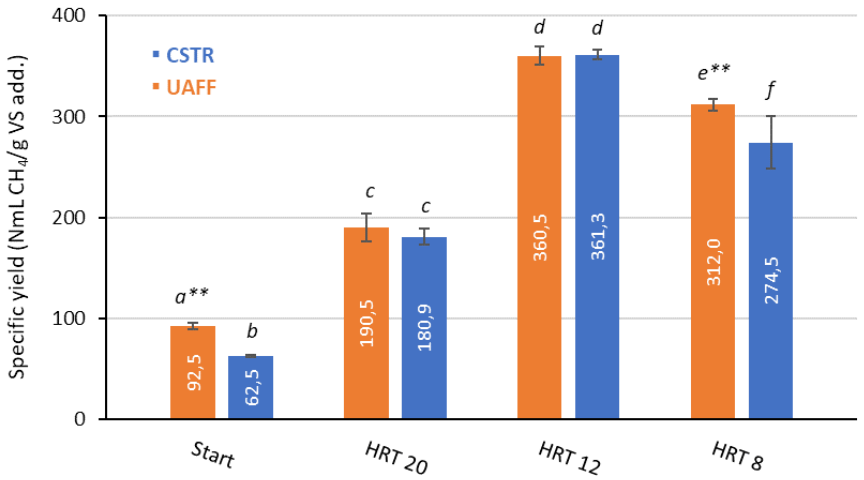

Figure 16. Methane yields obtained by conventional continuously stirred reactor (CSTR) and uplow anaerobic floating filter reactors (UAFF) during the start phase and operating with different hydraulic retention times (HRT).

B.4. CONCLUSIONS

Wastewaters obtained from the Ittica Caldoli – PP9's hatchery recirculation aquaculture system (RAS) were subjected to microfiltration and sedimentation, and the thickened sludges were anaerobically digested in batch assays for 36 days at 38°C in automatic methane production test system (AMPTS) and in bioreactor simulator system (BRS).
The anaerobic digestion process resulted proficient in the treatment of effluents from fish production despite the relevant dilution, low organic matter content and salts presence. The maximum biochemical methane potential (BMP), determined in batch lab-scale conditions, resulted in 564.2 NmL CH_4/g VS,

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which compared to animal waste could be considered a considerable high potential. In semi-continuous loading tests carried out at a pilot scale, the highest yield was achieved in brackish conditions, corresponding to 300.3 NL per kg of input VS, with the hydraulic retention time of 30 days and low OLR (0.28 g VS/L day). The AD process in marine conditions seemed to have a slower adaptation to salinity, but with an HRT of 20 days the yield raised to 241.2 NmL/g VS. The application of the retained biomass technology to the AD process could allow overcoming the high dilution of this type of wastewater, effectively lowering the HRT and reducing the risk of microbial biomass washout from the digester. In fact, with a new type of UAFF reactor, operating in batch conditions, the plastic filling media of the filtering bed showed a significant effect in terms of increase of the specific methane yield, achieving also a more rapid start-up than the anaerobic digestion process of brackish aquaculture effluents. In continuous load conditions, the operative parameters that determined the highest yields were represented by an HRT of 12 days and consequent OLR of 0.264 g VS/L day. With these settings, the specific yields resulted relevant (360.5 and 361.3 Nml CH_4/g VS, for CSTR and UAFF respectively). In these conditions also the CSTR digesters were capable of achieving an effective AD process, being an HRT of 12 days a fairly limited process time, representing a good compromise between a reduced wash out of anaerobic microorganisms and a relatively high organic load.

Lower HRT (8 days) determined a reduced specific yield, however UAFF resulted more efficient in these conditions, probably as a result of the highest efficiency in retaining anaerobic microorganisms despite the high hydraulic replacement rate. The UAFF digesters showed acceptable specific yields (312.0 Nml CH_4/g VS) even with these conditions.

By operating with an HRT of 8 days the specific yields were affected by a reduction of 13.3% in comparison with 12 days HRT, however the digestion volume would be reduced by 33.3%, with the same treated input: these results show that the implementation of UAFF reactors operating with reduced HRT would present investment advantages in case of a scale-up of the system to real operative conditions.

The graphics depicted in Figures 17 and 18 summarize the results of the experimental tests with a focus on the salinity of the effluent (i.e. brackish or marine). The brackish conditions allow achieving higher performances, with an average of 257.1 and 194.4 Nml CH_4/g VS, for brackish and marine respectively. Independently from the salinity level, the methane yields resulted higher adopting OLRs between 0.35 and 0.45 g VS/L day, and in correspondence with these values an optimal operative range could be defined for effective treatment of aquaculture effluents.

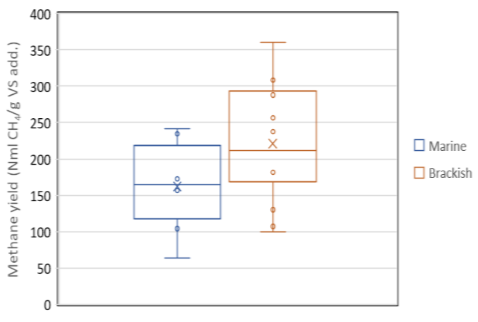

Figure 17. Box-and-whiskers plot of the specific yields in marine and brackish conditions from the different experimental tests of the Project.

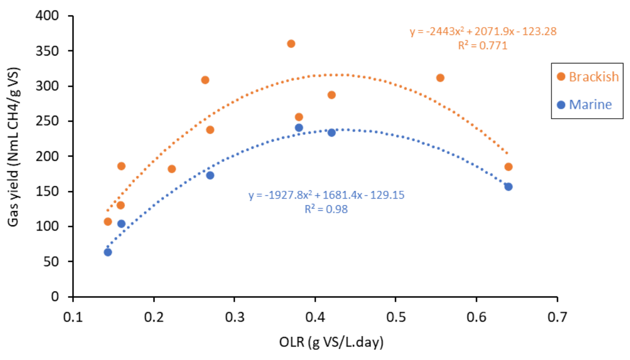

Figure 18. Evolution of the specific yield of methane in relation of the organic load of the reactors, for marine and brackish salinity conditions (synthesis of the results of the different tests performed in the frame of the Project).

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Reasons of discrepancies between planned and realized outputs
The first phase of the laboratory tests, performed with the bioreactor simulator system, was suspended in March 2020 and postponed to September 2020 due to the restrictions related to the COViD-19 sanitary emergency. However, the delay did not cause any discrepancies between the planned and realized output.
Impact of outputs underachievement on project results
The activities were recovered and were conducted according to the schedule, with a 6 months' delay, within the overall time of the project. The suspension of the activity did not cause any impact on the outputs or any underachievement in terms of the results of the project.
Additional results (additional outputs /results besides those foreseen in AF)
An anaerobic digester was tested, featuring the presence of an anaerobic filter composed of plastic media for the development of an anaerobic biofilter. The BRS system was modified with the introduction of the upflow floating filter, and its performance was tested in comparison with the conventional process. The good performance of the process, even with inocula from digesters fed with agricultural substrates, demonstrated the possibility of treating marine or brackish aquaculture effluents in co-digestion with more conventional feedstocks, with benefits in terms of energy yield.

C. DURABILITY AND TRANSFERABILITY OF THE PROJECT AND ITS RESULTS

How will the outputs and results be maintained and developed further after project end

The results of the project allow to define the main design, dimensional, and management criteria for anaerobic reactors operating with fish farm effluents, with high dilution, in brackish and marine salinity conditions. These results represent the starting point for the design and implementation at full scale of these plants in real fish farms. Furthermore, the research demonstrated that these effluents could be successfully used in existing plants operating mainly with livestock farming or agricultural feedstocks, with benefits in terms of energy output.

Possible aspects to be further studied after the end of the project are:

- In the RAS water recirculation systems implemented in hatcheries, the anaerobic digestion process would not determine a reduction of the total load of nitrogen, while achieving mineralization of part of organic nitrogen. An integrated RAS wastewater treatment line could feature a nitrogen removal line in sequence with anaerobic digestion, and biogas upgrading to biomethane to provide energy/fuel to sustain the process;
- Thermophilic temperature regime could enhance the performance of the AD process;
- Co-digestion with various feedstocks could represent a valid solution, for immediate implementation in existing biogas plants;
- Digestate, both in brackish and marine conditions, are characterized by a relevant content of organic substances, of nitrogen and phosphorous, and could find a suitable application in the cultivation of marine algae or microalgae. Even this aspect opens potential scenarios toward a complete circularity of fish farms;
- A further step could be represented by the investigation of the possibility of producing hydrogen by reforming methane, to be used to fuel cells for the production of continuous electricity;
- The technological treatment line (filtration, settling, thickening, anaerobic digestion) could be implemented for the management of wastewater from fattening sea cages; the research for new solutions for the recovery of wastewater from floating cages represents a challenge for the

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environmental sustainability of the entire production chain and it is a topic of extreme interest for future research projects in this field; - In order to reduce the plant and equipment costs, “simplified” reactors, i.e. characterized by flexible air-tight plastic bags with hydraulic mixing and sludge recirculation, could be tested for the treatment of fish farm effluents.
Availability of project results and outputs for general public and other stakeholders during the project life and after the project end During the project, 4 training courses were lectured to technicians, farmers, associations, veterinaries and operators of the sector in general. The training lessons entitled **“How wastewater of fish farm can produce energy, reducing pollution”** (Ostuni, Italy, September 2020**), “Renewable energy for sustainable aquaculture”** (on line, December 2020), and “**Energy Production from Fish Farm Wastes**” (Padova, Italy, November 2021; “**Energy production from Fish Farm Waste**“(Ostuni, Italy, May 2022) presented the results and outputs of the project to the producers, researchers, experts. The training materials were published also as deliverable 2.2 and distributed and will remain available even after the end of the project on the project website and intranet. This activity contributed to the reach of Programme output indicator CO44. Furthermore, the following scientific articles were published, and will be available after the end of the project, some in open-access mode: Da Borso F., Chiumenti A., Fait G., Mainardis M., Goi D. (2021) Biomethane Potential of Sludges from a Brackish Water Fish Hatchery. Applied Sciences, 11, 552. MDPI, Basel, Switzerland. https://doi.org/10.3390/app11020552. Chiumenti A., Owono Owono B., Fait G., Mainardis M., Goi D., Stella E., Da Borso F. (2021) Anaerobic Digestion of Brackish and High Salinity Aquaculture Sludges ISBN: 9781713833536; DOI: 10.13031/aim.202100230 American Society of Agricultural and Biological Engineers Annual International Meeting, ASABE 2021, 2021, Vol.2, p.719-724. Chiumenti A., Owono Owono B., Da Borso F. (2022). Upflow anaerobic floating filter (UAFF) for the anaerobic digestion of fish farm waste from brackish aquaculture. Submitted at the American Society of Agricultural and Biological Engineers Annual International Meeting, ASABE 2022.

D. CAPITALISATION OF RESULTS

Was the project able to capitalise or influence future calls or other projects? Please specify main results or output to be considered for future capitalisation action. The technological treatment line proposed in this project for intensive land-based hatcheries (filtration, settling, thickening, anaerobic digestion) could possibly be implemented for the treatment of wastewater from fattening sea cages. The research for novel solutions for the recovery of solid and liquid effluents from floating cages represents a challenge for the environmental sustainability of the entire production chain and represents a topic of extreme interest even for future capitalization actions. The development of biomass co-digestion systems, both with marine algae and conventional agricultural effluents, or simplified low-cost systems, represent other opportunities to be considered for future actions. **Are there any obstacles of legal or administrative nature that the project has encountered and which hampered cooperation? Is there any room to solve these obstacles?**

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No obstacles of legal or administrative nature have been encountered during the project that have limit the cooperation among people and institutions.

E. PARTNERSHIP COOPERATION

Partners active in WP 3.2.1, their experimental activities and contributions related to the report LP (WP 3 leader) and Ittica Caldoli PP9 were actively involved in this task. The following activities were performed in close cooperation with Ittica Caldoli (PP9): - the study of possible pre-treatment lines to concentrate the organic content in effluents; - the monitoring of the performance and set up of mechanic filtration by the drum filter available on the farm; - the collection of thickened fractions from the drum filter; - the performing of static settling tests, in controlled conditions, in order to achieve a further concentration of organic matter; - the sampling of thickened sludges to be destined for the anaerobic digestion process. This activity contributed to the reach of Programme output indicator CO01 and CO02. In cooperation with Bluefarm (subcontractor of LP) a study of literature regarding the environmental impact of cages and innovative systems for the recovery of solid particles released from cages was performed, with the aim of estimating the possible releases of effluents from new types of cages. In cooperation with PP4 and UniPD as subcontractor of PP4, a new protocol for the monitoring of energy consumption from PP9 hatchery and for the water heating in fish tanks was defined. Furthermore, LP strengthened the cooperation with researchers of DPIA - Dipartimento Politecnico di Ingegneria e Architettura of Udine University (Prof. Daniele Goi, Dr. Matia Mainardis), who were not directly involved in the project but gave support for the lab tests conducted with the AMPTS system and for chemical analyses of substrates. This activity contributed to the reach of Programme output indicator CO42.
Attractiveness of WP 3.2.1 Partners for other local/regional actors and involvement in the project activities Ittica Caldoli (PP9) is an established company with a solid relationship with the territory; therefore, it will be able to effectively spread the present outcomes to other stakeholders of the aquaculture sector even after the end of the project.
Added value given by the cooperation The cooperation with Ittica Caldoli (PP9) allowed having a better view of farm problems related to the management of effluents and water from the RAS. It was possible to determine the methane potential of these effluents, implementing standard lab procedures, but referring also to standard procedures implemented at the farm scale. Hence, the results of the laboratory and the following pilot-scale tests can be considered strictly related to real operative conditions. Thanks to this profitable cooperation, the assessment of the scale transfer of the process was more effective. The cooperation with DPIA of Udine University allowed the publication of two scientific articles and the presentation of data to International Conferences. Moreover, it resulted in strengthened knowledge on these topics and could be the base of future research projects.
Main problems encountered The main problems encountered during the project development were operative and due to the restrictions caused by the sanitary emergency experienced with the COVID-19 pandemic. Travelling, collection of feedstocks, and access to laboratories resulted impossible with a consequent obligation of

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suspending the experimental tests. The extension of the project allowed us to restore all the scheduled activities and achieve the project objectives without any limitations.
Links created by this project with other projects The synergies with other regional/national programs and local financial instruments were useful for the realization of AdriAquaNet activities within the task 3.2.1. Specifically, then link between this project and the working group coordinated by prof. Goi of DPIA of Udine University allowed to develop lab tests with the AMPTS system and chemical analyses of substrates. The UNIUD Group is recently proposing a new paradigm-project considering a partnership, as suggested by the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States (Sustainable Development Goals 6, 12, 17), with water&waste management companies and Friuli Venezia Giulia Regional Authority for water and waste services (AUSIR). The target is to organize a place in which students, scholars and people together are sharing, teaching and learning water&waste awareness within their own territory. A Technical Scientific Committee was instituted to reach in next years the objective to translate theories to practices and common understanding. Moreover, the link between this project and AQUASPACE Ecosystem Approach to making Space for Aquaculture EU Horizon 2020 coordinated by prof. Pastres (Bluefarm) allowed to implement and apply a computational model for estimating the possible release of effluents (dissolved inorganics, organic particulate, uneaten feed and faeces, organic carbon) from fish farm cages in order to use innovative systems for reducing the environmental impact. Finally, the link between this project and EU Horizon 2020 FutureEUAqua project that involves 32 partners, among which also PP4 and also the collaboration with the staff of University of Padova - engineers Marco Bullo and Matteo Lazzarin, helped to define a new protocol for the monitoring of energy consumption from PP9 hatchery and for the water heating in fish tanks. They agreed about a pilot study on a prototype, before starting the full-scale trial at Ittica Caldoli fish farm (Project Partner 9) in late spring 2021.
Possible cooperations in the future even without funding The cooperation with DPIA of Udine University allowed strengthening the group of researchers dealing with the theme of anaerobic digestion, with possible future exchanges and participation in research projects.

F. TARGET GROUPS INVOLVEMENT

Please list the main target groups that benefited from your WP project's achievements as inserted in the relevant Report Section in SIU that you will find on the left (the numbers are our project numbers). In few word provide further details on how they were able to make use of the outputs/ results of the project.

TARGET GROUPS	***Description***
SMEs (50)	One fish farm involved in the experimentation, 13 enterprises (Fanin, Bluefarm, Gas Clima Service, Skretting, RINA Service, INmare rehomare srl Gallipoli, Ittinsect srl, Panittica Italia Società Agricola srl, Acquacoltura Jonica, Azienda Agr. Eredi di B., Agris Sardegna, Acqua soc. Agr., Cromaris) participating directly to trainings in Padua and Ostuni, one enterprise (Sustainable Technologies – Italy) for the development of

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	the BRS system used for the anaerobic digestion of fish farm effluents followed our lessons.
Universities, technology transfer institutions, research institutions (10)	Scientific collaboration with the researchers of DPIA - Dipartimento Politecnico di Ingegneria e Architettura of Udine University (Prof. Daniele Goi, Dr. Matia Mainardis): support for the lab tests conducted with the AMPTS system and for chemical analyses of substrates.
NGOs, associations, innovation agencies, business incubators, cluster management bodies and networks (5)	https://webgate.ec.europa.eu/maritimeforum/en/node/7053 Italian Fish Farmers association (API) 3 Representatives of Order of the veterinarians Brindisi, Taranto and Lecce, 1 Aquarium Pula, 1 from AUL SS3 were present during our lessons.
Centers of R excellence (5)	Rappresentative persons of Centro di Ricerche per la Pesca e l'Acquacoltura, OISPA Tecnologia & Ricerca scrl, Stazione sperimentale per lo studio delle Risorse del Mare were involved in trainings in Ostuni.
Local, regional and national public authorities (10)	Representatives from Puglia Region, FVG Region, Veneto Region, MIPAAF, Ministry of Health, Interreg Programme Italy and Croatia, Croatian Chamber of Commerce
General public (1000)	Dissemination of results on the AAN project website and through radio or tv involved more than 3 million persons

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PART 2

A. CONTRIBUTION TO EUSAIR

Please provide a description of the project contribution to the EUSAIR in terms of synergy with the Strategy's pillars and alignment of implemented project's activities with the Action Plans and labelled projects.

The project directly involved researchers from University and public Institute, fish farms and hatcheries, enterprises (SMEs being part of the aquaculture business chain such as companies for feed producing, recycling wastes, fish food transforming), and different type of stakeholders (experts, general public, productive associations, policy) from Italy and Croatia in order to improve the competitiveness of the mariculture sector Adriatic Sea. The results of task 3.2.1 will ensure important positive impacts on innovation, economic development, job creation, and environmental sustainability. The project approach and outcomes can be transferred to other territories of the EUSAIR macro region, thus multiplying the positive effects of project outputs. In this case, the innovative models and techniques for limiting the pollutant emissions and dispersions from fish cages and the new systems to treat wastewater from intensive fish farms, that were tested during the project and are proposed for an application in sea bass/bream intensive farming, will contribute to reduce the marine pollution and maintain the marine biodiversity, as required by EUSAIR action plan that identifies aquaculture as a key sector in the blue economy of Italy, Croatia and Greece, having the potentiality to play a pivotal role in the entire area.

B. CONTRIBUTION TO HORIZONTAL PRINCIPLES

Please provide a description of the project contribution to the horizontal principles of equality between men and women, non-discrimination and sustainable development.

The project engaged technical and administrative staff based on personal characteristics, complying with the equal opportunities and without discriminations, such as gender, race, nationality, ethnic origin, religion or belief, disability, age or sexual orientation. The employment relationship was based on the principle of equal opportunity and fair treatment, including type of contract, wages and benefits, working conditions and terms of employment, access to training, promotion, and termination of employment as for any other Italian or Croatian staff hired. The staff and external services involved were formed without any kind if discriminations based on personal characteristics, genre, age, belief, race, nationality, ethnic, religion and belief, sexual orientation, etc. Several foreign doctoral students of UNIUD were involved also in the project activities (in the task 3.2.1 one doctoral student from Equatorial Guinea was actively involved in the project activities and participated in the report writing). The project activities and outcomes can be considered as contribution to sustainable development and within the task 3.2.1 in particularly, in terms of environmental, economic and social sustainability. New efficient technologies for the management of fish farm waste effluents and innovative systems for energy saving were tested and improved, in order to reduce the environmental impact of sea bass/bream farms. The application of these implemented technologies in sea bass/bream farming in the Adriatic area will also ensure a better productivity and profitability of fish farm, providing permanent employment opportunities and increase the sustainability of the aquaculture sector.

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C. COMMUNICATION ACTIVITIES

Please refer to the Final Communication Report template and provide a summary on the main achievements trying also to identify which were the most successful communication tools in reaching general public/decision makers/other target groups.

All the activities performed to reach the present DL have been documented with photos and videos taken by LP and PP9 communication specialists. The material has been uploaded on the Intranet website of the project. Some of the materials was used to produce this report (see above), and to produce communication materials. The aforementioned activities have been presented at the training events held in Padua, Ostuni and online. During the final conference in Zadar (3 June 2022) and Udine (20 June 2022) a summary of the most important results have been presented by LP, PP4, and PP9 staff. Numerous reports, meetings, brochures, training courses, conferences, a website and a YouTube channel have been produced to communicate the results.

D. NATURA 2000

Please describe, if it is the case, measures foreseen and implemented by the project:

a) In case the project involved Natura 2000 sites, describe what measure the project envisaged and implemented to avoid any negative impact: No Natura 2000 sites are included in the areas where the project activities have been carried out; therefore, no measures have been envisaged and implemented during the project in order to avoid negative impacts.
b) In case the project had a positive effect on Natura 2000 sites, please describe which measure the project has foreseen and implemented in order to reach a direct or indirect positive impact: No Natura 2000 sites are included in the areas where the project activities have been carried out; therefore, no measures have been foreseen and implemented during the project in order to reach positive impacts.

E. TYPES OF ACTIONS ADDRESSED (as defined in the Cooperation Programme)

These are our primary objective's types of actions, that we addressed by the Project:

Specific Objectives	***Types of action***	***the most relevant one within the SO addressed by your project***
1.1 Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area	*Joint projects and actions aimed at creating platforms, networks and at supporting exchange of good practices in order to enhance the knowledge transfer and capitalization of achieved results in the field of blue economy*	***X***
	Actions aimed at cluster cooperation, joint pilot initiatives in order to boost the creation of marketable innovative processes and products, in the field of blue economy	***X***

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F. TYPES OF OUTPUTS PRODUCED

Specify the types of outputs generated by your activity that are reported here and provide a brief description

Output typology	Description
Trainings	4 training courses in Italy and Croatia regarding the potential of biogas technology applied to wastewater from fish farms and energy recovery for a reduction of fish farms environmental impact have been performed during the project.
Monitoring systems	N.A.
SMEs clusters	Potential collaboration and exchange of work and resources among enterprises involved in the aquaculture business chain such as fish farms and companies for aquafeeds producing and waste recycling were established. The innovative techniques and systems implemented during the project within the task 3.2.1. can be applied in other Italian and/or Croatian fish farms and facilities. The cross border production chain that involves Italian hatcheries, which grow sea bass and sea bream fingerlings and juveniles, and Croatian on-growing sea cages-based farms, which than exported the fish to the Italian market, was implemented thanks to the project training courses and events.
New networks	New collaborations among project partners, subcontractors and researchers of Udine University were developed during the project in order to achieve the task 3.2.1 objectives. Moreover, an active cooperation among researchers of LP and fish farmers of PP9 was developed so as to improve the interest of entrepreneurs for R&D and innovation as well as allow the project to respond to their needs.
Platforms	N.A.
Adaptation plan	N.A.
Building renovation	N.A.
Others (please specify)	N.A.

G. TYPOLOGY OF IMPACTS

Please indicate what type of impact(s) your project has had. You can choose more than one answer. For each tangible impact selected, please provide a concrete example from your project, where possible supported by quantitative information.

TANGIBLE IMPACTS

Tangible impacts	Example/ quantitative information
Improved access to services	N.A.
Cost savings	The design and implementation of photovoltaic plant at PP9 within the task 3.2.1. will ensure the reduction of costs for fish production.
Time savings	N.A.

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Reduced energy consumption	The design and implementation of biogas production plant within the task 3.2.1. will ensure the energy saving and production of renewable energy.
Reduced environmental impact	The application of the innovative and smart techniques and systems, which were developed and implemented during the project within the task 3.2.1., in sea bass/bream intensive farms in the Adriatic area will effectively limit the pollutant emissions and dispersions from fish cages as well as will ensure an adequate treating of wastewater, contributing to reduce the marine pollution and maintain the marine biodiversity.
(Man-made, natural) risk reduction	N.A.
Business development	The application of the innovative and smart techniques and systems for pollutant reduction, waste management, and energy saving, which were developed and implemented during the project within the task 3.2.1., in sea bass/bream intensive farms in the Adriatic area will ensure a better productivity and more eco-compatible productions that will be more appreciated by the consumers, increasing the profitability of the mariculture sector.
Job creation	The application of the innovative and smart techniques and systems developed and implemented during the project within the task 3.2.1. in sea bass/bream intensive farms in the Adriatic area and the consequent higher productivity and profitability of the aquaculture sector can provide permanent employment opportunities to costal populations of both sides of the Adriatic sea.
Improved competitiveness	The increased productivity and profitability of the aquaculture sector in the Adriatic area through the application of the innovative and smart techniques and systems for pollutant reduction, waste management, and energy saving, which were developed and implemented during the project within the task 3.2.1., in sea bass/bream intensive farms in the Adriatic area will ensure an increased competitiveness of SMEs on regional and international markets.
Other tangible impacts (specify)	N.A.

INTANGIBLE IMPACTS

Intangible impacts	**Example/quantitative information**
Building institutional capacity	N.A.

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Raising awareness	The project has stimulated the attention of fish farmers regarding the issues of energy saving and reduction of the farm environmental impacts through the study of new techniques for the production of renewable energy and the treatment of wastewater from hatcheries and sea cages, so to improve the sustainability of Mediterranean aquaculture.
Changing attitudes and behaviour	The project provide to fish farmers new techniques for the production of renewable energy and the treatment of wastewater to be applied in hatcheries and sea plants, so to improve the sustainability of Mediterranean aquaculture and consequently the competitiveness of sector.
Influencing policies	N.A.
Improving social cohesion	N.A.
Leveraging synergies	The project lead to the strengthening of relations between Italian and Croatian research groups, as well as between universities or centres of excellence and fish farmers. This collaboration may be exploited in the future for the drafting and implementation of new research projects aimed at improving aquaculture farming systems and waste/energy management in fish farms.
Other intangible impacts (Specify)	N.A.

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