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1 Introduction

The iFishIENCI project contributes to the consolidation of a circular and blue bioeconomy by proposing and qualifying new organic value chains for feed and valorisation of by-products from fish farming. Particularly, the project identifies and assesses new value chains for the valorisation of sludge and wastewater from different production systems, looking into the alternatives to recirculate these waste streams as nutrient sources for algae- and yeast-based feed production. While feed composition and feeding strategies are optimized, unnecessary losses from the farms are minimized leading to notable reductions of sludge volume. In addition, the recirculation of nutrients within the value chain is part of the core circular practices proposed and developed in iFishIENCi (Figure 1).

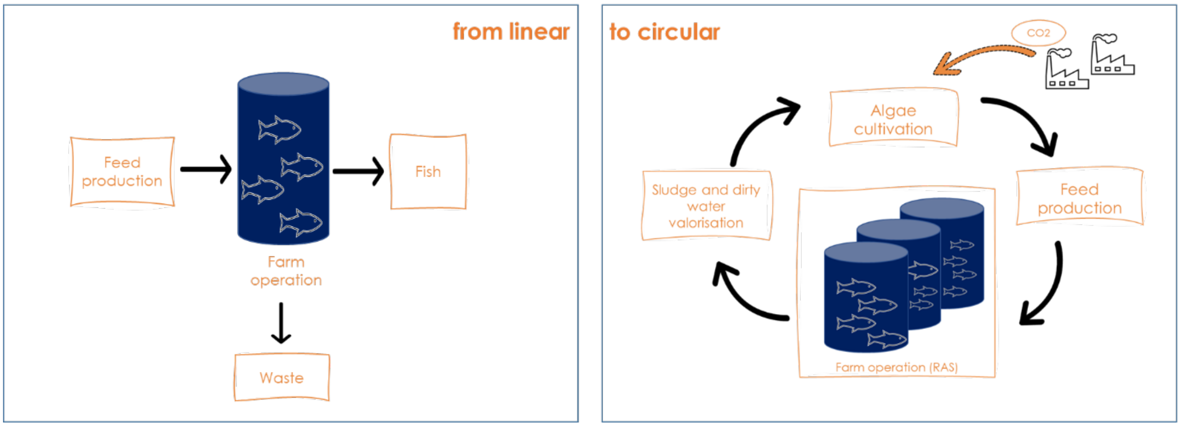


Figure 1 Moving from linear to circular approach in iFishIENCi

The project addresses the circularity in the context of WP4, where Task 4.1 evaluates how well the proposed value chains perform under a circular perspective. During the first project period, detailed research revealed that there were no standards to assess circularity in aquaculture and consequently the development of a methodology was deemed necessary. The main circularity attributes of the iFishIENCi concept were investigated to define the corresponding indicators. In this sense, the circularity of nitrogen as key nutrient for fish nutrition (protein) appeared to be the most promising parameter which represents best how efficient the technologies and processes were towards a zero-waste and circular fish farming. Moreover, the circularity of nutrients is also a good opportunity for the sector, being identified as a circular practice in the new Circular Economy Action Plan (COM(2020) 98 final). Aligned with this, we developed a methodology which delivers 4 indicators adapted and easy to interpret which inform on the circularity under a holistic approach and life cycle perspective.

This deliverable aims to present the results obtained from the circularity assessment by applying the methodology to the project case studies and to explain how the development of this work has allowed us to elaborate on recommendations for policy makers. Finally, some conclusions and recommendations are provided to pave the way for a more circular aquaculture and circularity assessment.

2 Circularity assessment

The review of different methodologies and approaches during the previous project period, helped us to conclude the need of developing a new method to facilitate the evaluation of aquaculture processes. Although circularity can be addressed in a relatively simple way for the technical cycles and sectors associated, available frameworks identified during the first project period did not provide guidelines for the circularity evaluation of biological flows, such as those involved in aquaculture.

When the Material Circular Indicator (MCI) developed from Ellen Macarthur[1] was analysed, this had not been revised yet to include biological materials. Despite offering indicators to measure circularity under a life cycle perspective, MCI was not applicable for food industry in that moment. After developing our own methodology in the context of the project, the MCI was revised to include biological materials and sustained production concept, which basically means that the extraction of natural materials aims to maximise the regeneration of natural system. The revised version included the composting and energy recovery as end-of-life practices, but it was still insufficient to be fully used for the evaluation of the circularity in IFishIENCi project. In any case, both approaches, the revised MCI and iFishIENCi project, entail the same system thinking as both frameworks consider the origin of the biological materials and how to return them to the biological cycle as accessible nutrients.

On the other hand, the European PEF initiative [2]and associated Circular Footprint formula (CFF) were also investigated to prospect the possibilities to measure circularity for aquaculture. This approach allows calculating the environmental attributes of the end-of-life stages, combining the assessment of the material, energy, and disposal practices. CFF provided the evaluation of the environmental implications of the end-of-life scenarios through any of the recommended LCA impact categories. Given that iFishIENCi seeks to develop new innovative valorisation routes and new feed ingredients (materials), CFF did not seem to be the most appropriate framework as it did not provide factors for the project processes. Moreover, the environmental footprint is addressed within Task 4.3 which aims at delivering a LCA study of the iFishIENCi concept. In this sense, results from the LCA allow us to interpret the circularity with complementary indicators which inform on the sustainability profile.

Concurrently with this project, the International Organisation for Standardization, ISO, has been working in the context of Circular Economy to develop frameworks, guidance, supporting tools and requirements for the implementation of activities of all involved organizations, to maximize the contribution to Sustainable Development, developing in 2019 some transversal standards related to circular economy. Particularly ISO 59020, due to be published in early 2024, will provide a framework for organizations to measure and assess circularity, applicable to multiple levels, from regional to product level.

To conclude, it is of relevance to mention that all above-mentioned frameworks are developed under a general approach, without considering any specific production sector, and finally they seem to be a bit insufficient to address the project innovation particularities. Therefore, during the previous project period, the project team confirmed not only the obligation to develop and test a customized methodology to respond to the needs of assessing specific circularity indicators but also indicators that may serve for the aquaculture sector in general and beyond the project.

[1] Available at https://emf.thirdlight.com/link/yybss1obhtdv-ub419h/@/preview/1?o
[2] https://green-business.ec.europa.eu/environmental-footprint-methods_en

The methodology developed consist of the definition of 4 key indicators:

- Linear Flow Index, LFI, represents the linearity of the feed formulation, through the quantification of virgin nitrogen with respect to the total nitrogen in the feed. LFI=1 means that the feed is totally linear. Ingredients not elaborated from the valorisation routes developed in the project are considered as linear, for example fish meal.
- Material Circularity Indicator, MCI, is defined in accordance with the LFI and the utility. According to the methodology published by Ellen Macarthur (2015), the utility has two main components: the lifetime of the product and the intensity of use. The lifetime is a variable for measuring durability. As feed is not restored into the market after repairing or maintenance, it cannot be considered as a technical product and lifetime concept is not applicable under this approach. The intensity reflects the extent to which a product is used to its full capacity. In the context of iFishCIENCI, the ratio is represented by the Nitrogen assimilation efficiency (NE).
- Nitrogen Waste Indicator, NWI, provides information on the nutrient recycling efficiency within the system boundaries (until farm-gate). It considers the total unrecoverable nitrogen compared with the total nitrogen contained in the innovative feed.
- Zero waste indicator, ZWI, assess the zero-waste attributes even if alga-based products are used out of aquaculture sector, enabling the recirculation of nutrients and therefore the circularity with resources that are sourced from waste streams. This indicator gives us information on how restorative the nutrient waste stream can be, considering the algae efficiency cultivation and total nutrients not recovered.

Specific formulas were consequently defined and later protected in the context of the Key Exploitable Result (KER) regarding the circularity assessment. They are used for the calculation of indicators, but they are not included in this deliverable to avoid any negative effect on the exploitation of the project result.

This deliverable entails the assessment of circularity by quantifying indicators that allow the evaluation of different trials and demonstrations. Regarding Task 4.2 scope, the circularity assessment was conducted for the experiments where the following criteria were met:

- Both streams, sludge and wastewater can be feasibly collected for valorisation in close systems (RAS)
- Microalgae or yeast are incorporated into the provided feed
- Characterization of waste sludge (effluent from the drum filter or other mechanical filtration unit) and dirty water (the water flow from the production tanks to the water treatment unit, before any treatment) (information on nutrient content) is available

Despite both streams, outlet water and sludge are normally collected in commercial RAS farms (water is recirculated and sludge is managed as biological waste), only when nutrients are recovered and captured for a new value chain, circularity are considered in the context of iFishIENCi and these experiments are assessed through the circularity assessment. The figure below shows the decision tree developed aiming to select the demonstrations trials that target the circularity principles of the project and where the monitoring and traceability of nutrients through the value chain are possible. As can be deduced, the circularity assessment serves to address the demonstrators focused on recirculated systems developed within task 3.4.

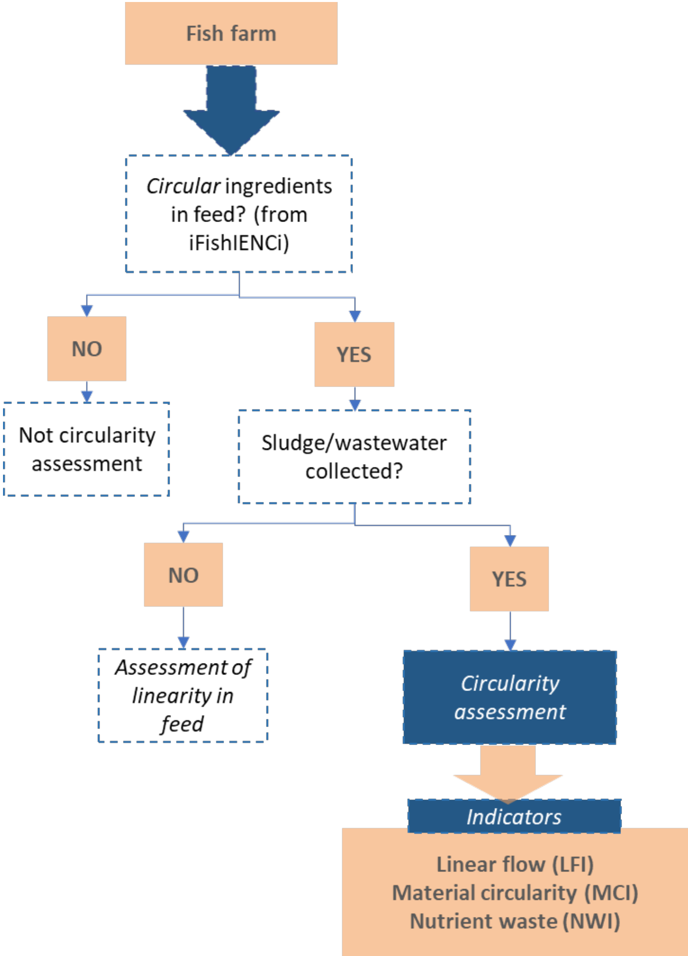


Figure 2 Decision tree for circularity assessment

Apart from ingredient, algae were also tested as antioxidant Nanno extract (supplement) in the feed formula for some trials (RAS2_194 and IFN01_LC). However, we do not include it into the assessment since the integration of the nano extract in the feed formula was insignificant therefore feed with "nanno extract" is considered as linear feed.

On the other hand, waste streams from flow-through demo site were collected, characterized, and tested for algae growth in task 1.5. Nevertheless, the circularity of this system is not evaluated as they did not feed fish with new ingredients from the project.

Regarding open systems, the circularity assessment enables the evaluation of the feed in terms of circularity of the ingredients. However, the rest of indicators cannot be normally calculated as waste streams were neither collected nor valorised. Despite this limitation, a theoretical analysis is included in section 3.6 to provide a comprehensive approach for a scenario that could be achieved according to some progress made for sludge collection in open systems.

In summary, the following table shows the experiments considered for the circularity evaluation, together with a brief description of the scenarios defined for the study of indicators:

Trial/Demo	Description of valorisation trials (WP1)	Considerations for the circularity assessment
RAS1_188: Rainbow trout, feed: algae (*Nannochloropsis gaditana*[3]*1) 30% inclusion*	Dirty water used for growing algae Sludge and medium ingredient[4] from sludge directly used for algae cultivation	Scenario a) both streams, dirty water and sludge are collected but only dirty water is valorised as substrate for algae cultivation
		Scenario b) both streams, dirty water, and sludge (after autoclave) are collected and valorised for algae cultivation
RAS5_243: Rainbow trout, feed: yeast (*Candida utilis)* 30% inclusion	Waste streams not used for growing yeast (dirty water nor sludge) but for testing algae growth.	Scenario a) both streams, dirty water and sludge are collected but only dirty water is valorised as substrate for algae cultivation
		Scenario b) both streams, dirty water, and sludge (through the production of medium ingredient) are collected and valorised for algae cultivation
RAS6_275: Rainbow trout, feed: optimised meal diets with *Nannochloropsis gaditana* and *Candida utilis* (5% inclusion) and different levels of pigment (Astaxanthin)	Dirty water was used for testing yeast growth. Sludge was used as substrate for yeast and additionally medium ingredient obtained from sludge was used for both microorganisms; algae and yeast cultivation	Scenario a) outlet water and medium ingredient from sludge (chemically treated) are valorised through cultivation of algae.
		Scenario b) valorisation of unconcentrated sludge[5] with yeast
		Scenario c) valorisation of medium ingredient with yeast
IFN03: African Catfish, feed: 10 and 20% of *Candida utilis*	No waste streams were used for valorisation experiments in WP1 due to timing constrains (IFN03 started when T1.5 was about to finish)	Scenario a) 10% inclusion Candida Only water is valorised through algae cultivation
		Scenario b) 20% inclusion Candida Only water is valorised through algae cultivation
		Scenario c) 20% inclusion Candida Only sludge (through medium ingredient-enzymatic process) valorised through yeast cultivation

[3] *Nannochloropsis gaditana*, renamed *Microchloropsis gaditana* in recent years.
[4] Medium ingredient refers to nutrients extracted from concentrated sludge by enzymatic or chemical hydrolysis. Medium Ingredient are used for microalgae and yeast cultivation in the context of Task 1.5.
[5] Raw sludge or unconcentrated sludge refers sludge from RAS system with 0.5-4.3% dry matter.

3 Results

3.1 Circularity of RAS1_188

In this trial, 30% inclusion rate of the *Nannochloropsis gaditana* was formulated in feed and delivered to Rainbow trout. The microalgae was produced at the National Algaepilot in Mongstad and disrupted by bead-milling to ensure better digestibility. Waste streams retrieved from both Control and "Nanno" diets were collected, characterised, and used for valorisation experiments in the context of task 1.5.

This trial was taken as reference to validate the methodology developed during the last project period. However, the circularity assessment of this trial was developed considering default values sourced from literature as the samples had not been fully analysed and valorisation experiments had not been concluded when D4.4 was delivered. Now, this assessment aims at updating the results included in the previous circularity report, once all the parameters were properly calculated with primary data.

In the context of task 1.5, unconcentrated sludge from RAS1_188 was the only waste stream used for testing if algae could grow, but further experiments were not run with sludge. To provide a comprehensive analysis of the potential circularity of this system, the assessment addresses the following scenarios:

- Scenario a) outlet water is used to grow algae, but sludge is not valorised
- Scenario b) both streams, outlet water and sludge are valorised through algae cultivation

As part of the valorisation experiments in T1.5, algae growth was observed in wells plates with sludge after autoclaving. Therefore, scenario b is defined as a viable and additional scenario for the analysis of the circularity. The medium ingredient obtained through enzymatic treatment of sludge was not successful for algae cultivation, and consequently this is neither considered nor evaluated as a feasible circular route.

To define and assess the scenario b, two principal assumptions are made. Firstly, it is assumed that autoclaving does not vary the nitrogen availability of the sludge. Secondly, the nitrogen capture efficiency by algae when (diluted) sludge is used as substrate is assumed to be the same as the efficiency obtained when water is used as substrate.

Table below shows the updated figures for both, scenarios, a, and b. These parameters allow evaluating the circularity and interpreting the system also in terms of zero-waste.

Table 1 Parameters from Trial RAS 188 1

PARAMETERS	**Data (from Trial RAS 188 1)**
Total feed provided (kg DW):	5.31
Total nitrogen in feed (**M**) (kg DW)	0.36
Virgin nitrogen in feed (**V**) (kg DW)*	0.22
Eaten (ingested) feed (%)	99.02
Nitrogen assimilation efficiency of new feed developed in iFishIENCi (**NE**)	95.21
Nitrogen assimilation efficiency of conventional feed with similar properties (**NE_{av}**)	96.41

PARAMETERS	Data (calculated from T1.5)	
Nitrogen content in sludge[6] (mg/kg DW)):	59,021	
*Total nitrogen sludge (kg DW) (**C´**$_{RS}$)*	0.0621	
*Nitrogen content in dirty water (kg DW) (**C´**$_{RW}$)*	0.18	
PARAMETERS	**Data (calculated for Scenario a)**	**Data (calculated for Scenario b)**
Nitrogen recycling efficiency for sludge (Ecs)	*0%*	*100%*
Nitrogen recycling efficiency for dirty water (Ecw)	*100%*	*100%*
Nitrogen recycling efficiency for algae cultivation (Ef)	*82.8%*	*82.8%*
PARAMETERS	**Data (calculated for scenario a)**	**Data (calculated for scenario b)**
Nitrogen not recovered (W0) (kg DW)	*0*	*0*
Nitrogen in sludge not recovered for the new value chain (Wcs) (kg DW)	*0.0621*	*0*
Nitrogen in dirty water not recovered for the new value chain (Wcw) (kg DW)	*0*	*0*
Nitrogen not taken by algae (WF) (kg DW)	*0.0615*	*0.0722*

Given the parameters obtained and presented in the previous above, Linear Flow Index (LFI), Material Circular Indicator (MCI), Nitrogen Waste Indicator (NWI) and Zero Waste Index (ZWI) are calculated as indicators.

Table 2 Circularity assessment of RAS1_188, scenario a)

INDICATORS	RESULT
Linear Flow Index (LFI)	**0.60**
Material Circular Indicator (MCI)	**0.45**
Nitrogen Waste Indicator (NWI)	**0.34**

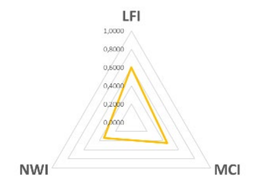
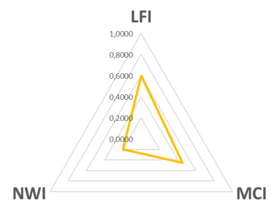


Table 3 Circularity assessment of RAS1_188, scenario b)

INDICATORS	RESULT
Linear Flow Index (LFI)	**0.60**
Material Circular Indicator (MCI)	**0.45**
Nitrogen Waste Indicator (NWI)	**0.20**



[6] Total elemental Nitrogen

Both scenarios equally perform in terms of LFI and MCI, however scenario b provides a better performance in terms of NWI as sludge is considered suitable and used for algae cultivation. In terms of zero waste indicator, results are provided in the following table for both scenarios of RAS1_188:

Table 4 Zero waste indicator of RAS1_188

	RAS1_188, scenario a)	RAS1_188 scenario b)
INDICATORS	**RESULT**	**RESULT**
Zero Waste Index (ZWI)	**0.79**	**1.00**

As both scenarios, a and b, are referring to the same digestibility trial, the indicators of LFI and MCI are the same since they are directly dependent of the eaten feed and assimilation efficiency. The main difference is observed in the indicators of waste generation, being scenario b the most beneficial in terms of waste valorisation. As all nitrogen would be available for microorganism cultivation in scenario b, no nitrogen waste would be raised from the valorisation and the system would work totally under a zero-waste approach (equal to 1).

3.2 Circularity of RAS5_243

Outlet water and sludge from faeces collector from Rainbow trout were collected in the context of Task 1.5. Waste streams retrieved from Control diet and waste retrieved from Candida powder diets were collected in triplicate and delivered to NORCE (water, sludge) and LEITAT (sludge). In this trial, 30% inclusion rate of the *Candida utilis* was formulated in feed.

Waste streams from this RAS trial were used to test the algae growth but not the Candida cultivation. Although the route is not completely circular as different extractive species are cultivated, the valorisation route maintains the principle of nutrient recirculation, therefore it is considered in the circularity assessment.

Experiments within T1.5 were run to valorise the outlet water and sludge enzymatically treated to obtain medium ingredient. Both streams were demonstrated to be feasible for growth algae in line with the well pallets test results. As further valorisation experiments, only outlet water was tested as substrate for microalgae in photobioreactor.

In line with the assessment of RAS1_188, two scenarios are defined for RAS5_243:

- Scenario a) outlet water is used to growth algae, but sludge is no valorised.
- Scenario b) both streams, outlet water and medium ingredient from sludge are valorised.

The enzymatic treatment has been considered as the reference process to obtain medium ingredient, with the corresponding nitrogen recovery efficiency studied in WP1. In linear with RAS1_188, efficiency of the nitrogen capture by algae has been assumed to be the same as the efficiency calculated with the outlet water. Next table summarises the parameters for the circularity assessment.

Table 5 Parameters from Trial RAS5_243- Scenario a)

PARAMETERS	**Data (from Trial RAS5 243)**	
Total feed provided (kg DW):	5.91	
Total nitrogen in feed (**M**) (kg DW)	0.43	
Virgin nitrogen in feed (**V**) (kg DW)*	0.23	
Eaten (ingested) feed (%)	99.74	
Nitrogen assimilation efficiency of new feed developed in iFishIENCi (**NE**)	95.62%	
Nitrogen assimilation efficiency of conventional feed with similar properties (**NE_{av}**)	96.56%	
PARAMETERS	**Data (calculated from T1.5)**	
Nitrogen content in sludge[7] (mg/kg DW)):	16,055	
*Total nitrogen sludge (kg DW) (**C'_{RS}**)*	0.02	
*Nitrogen content in dirty water (kg DW) (**C'_{RW}**)*	0.11	
PARAMETERS	**Data (calculated for Scenario a)**	**Data (calculated for Scenario b)**
Nitrogen recycling efficiency for sludge (Ecs)	*0%*	*36%*
Nitrogen recycling efficiency for dirty water (Ecw)	*100%*	*100%*
Nitrogen recycling efficiency for algae cultivation (Ef)	*92%*	*92%*
PARAMETERS	**Data (calculated for scenario a)**	**Data (calculated for scenario b)**
Nitrogen not recovered (W0) (kg DW)	*0*	*0*
Nitrogen in sludge not recovered for the new value chain (Wcs) (kg DW)	*0.0189*	*0.0121*
Nitrogen in dirty water not recovered for the new value chain (Wcw) (kg DW)	*0*	*0*
Nitrogen not taken by algae (WF) (kg DW)	*0.0088*	*0.0093*

Given the parameters presented above, the circularity is calculated through the 3 indicators- LFI, MCI and NWI, and presented in the following table. of these nutrients.

Table 6 Circularity assessment of RAS5_243, scenario a)

INDICATORS	**RESULT**
Linear Flow Index (LFI)	**0.54**
Material Circular Indicator (MCI)	**0.51**
Nitrogen Waste Indicator (NWI)	**0.06**

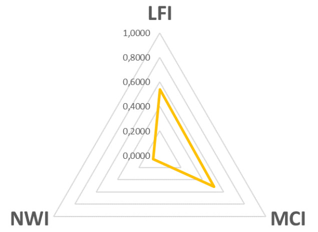
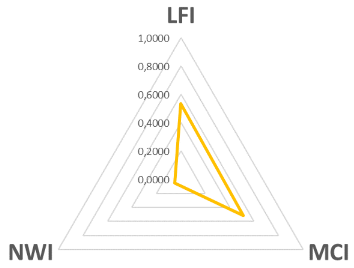


Table 7 Circularity assessment of RAS5_243, scenario b)

[7] Total elemental Nitrogen

INDICATORS	RESULT
Linear Flow Index (LFI)	**0.54**
Material Circular Indicator (MCI)	**0.51**
Nitrogen Waste Indicator (NWI)	**0.05**



LFI and MCI are the same as they are basically referring to the same feed properties. However, NWI is better for scenario b than a, as nutrients in sludge are partially recovered when this is treated with an enzymatic process.

Results for ZWI are also provided, representing the attributes of the system to recover nutrients, independent of the final use.

Table 8 Zero waste indicator of RAS5_243

	RAS5_243 scenario a)	RAS5_243 scenario b)
INDICATORS	**RESULT**	**RESULT**
Zero Waste Index (ZWI)	**0.81**	**0.89**

ZWI is comparatively better in scenario b, where sludge is treated. However not all nitrogen would be available for microorganism cultivation, so ZWI are close but not equal to 1.

3.3 Circularity of RAS6_275

Waste streams (water and sludge) from rainbow trout fed with optimised meal diets incorporating *Nannochloropsis gaditana* and *Candida utilis* (5% inclusion rate) were collected and characterized within Task 1.5. The circularity assessment addresses the scenarios concerning the following diets:

-Candida diet (5% substituted for FM; 40 mg/kg Astaxanthin),
-Nanno diet 1 high pigment (5% substituted for FM; 40 mg/kg Astaxanthin),

Particularly, three different valorisation routes were tested in WP1, therefore the following scenarios are defined for the circularity assessment:

- Scenario a) outlet water and medium ingredient from sludge (chemically treated) are valorised through algae cultivation
- Scenario b) valorisation of unconcentrated sludge for yeast cultivation, no valorisation of water
- Scenario c) valorisation of medium ingredient from sludge for yeast cultivation, outlet water is not valorised

Regarding scenario a, results from T1.5 were only obtained for algae cultivation with Medium Ingredient, but outlet water is assumed to be feasible for algae cultivation as well, since nutrients are highly diluted. Generally, for this trial, sludge was characterized according to the diet (data on nitrogen content is available for sludge from yeast and algae diets, separately), but the valorisation tests did not provide differentiated results for each ingredient.

Aiming at providing a circularity evaluation, we perform the analysis when waste streams from Nanno-diets are assumed to be valorised for algae cultivation and waste streams from Candida-diets are assumed to be valorised as substrate for yeast cultivation. The tables below show the parameters calculated and used for the circularity assessment:

Table 9 Parameters from Trial RAS6_275- Scenario a) (Nanno- diet)

PARAMETERS	**Data (from Trial RAS6_275)- Nanno diet**	**Data (from Trial RAS6_275)- Candida diet**	
Total feed provided (kg DW):	7.72	8.05	
Total nitrogen in feed (**M**) (kg DW)	0.613	0.609	
Virgin nitrogen in feed (**V**) (kg DW)*	0.579	0.564	
Eaten (ingested) feed (%)	0.95	0.99	
Nitrogen assimilation efficiency of new feed developed in iFishIENCi (**NE**)	95.21	95.62	
Nitrogen assimilation efficiency of conventional feed with similar properties (**NE_{av}**)	96.41	96.56	
PARAMETERS	**Data (calculated for scenario a)**	**Data (calculated for scenario b y c)**	
Nitrogen content in sludge[8] (mg/kg DW)):	54,309	39,959	
*Total nitrogen sludge (kg DW) (**C'_{RS}**)*	0.05	0.04	
*Nitrogen content in dirty water (kg DW) (**C'_{RW}**)*	0.31	0.23	
PARAMETERS	**Data (calculated for scenario a)**	**Data (calculated for scenario b)**	**Data (calculated for scenario c)**
Nitrogen recycling efficiency for sludge (Ecs)	46%	100%	36%
Nitrogen recycling efficiency for dirty water (Ecw)	100%	0%	0%
Nitrogen recycling efficiency for algae/yeast cultivation (Ef)	75.6%	0%	0%
PARAMETERS	**Data (calculated for scenario a)**	**Data (calculated for scenario b)**	**Data (calculated for scenario c)**
Nitrogen not recovered (W0) (kg DW)	0	0	0
Nitrogen in sludge not recovered for the new value chain (Wcs) (kg DW)	0.0293	0	0.0256
Nitrogen in dirty water not recovered for the new value chain (Wcw) (kg DW)	0	0.2296	0.2296
Nitrogen not taken by algae/yeast (WF) (kg DW)	0.0061	0.04	0.0144

Table 10 Circularity assessment of RAS6_275- Scenario a) (Nanno- diet)

[8] Total elemental Nitrogen

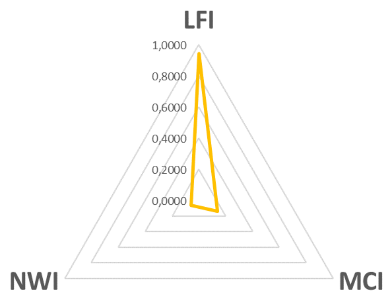
INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.94**	
Material Circular Indicator (MCI)	**0.14**	
Nitrogen Waste Indicator (NWI)	**0.06**	

Table 11 Circularity assessment of RAS6_275- Scenario b) (sludge candida- diet)

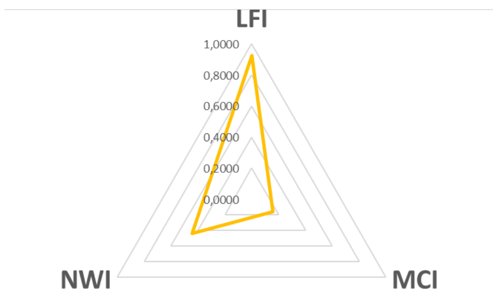
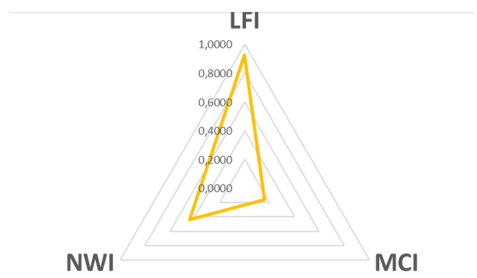
INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.92**	
Material Circular Indicator (MCI)	**0.16**	
Nitrogen Waste Indicator (NWI)	**0.44**	

Table 12 Circularity assessment of RAS6_275- Scenario c) (Medium ingredient sludge candida- diet)

INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.92**	
Material Circular Indicator (MCI)	**0.16**	
Nitrogen Waste Indicator (NWI)	**0.44**	

As scenarios b and c are referring to the same feed formulation, LFI and MCI have the same results. Although, the inclusion rate is 5% for all formulas, MCI of scenario a is worse than b and c. The reason underlying this result is that the utility is higher when fish are feed with *Candida* in comparison with *Nannochloropsis* when both formulas are compared with their corresponding controls in terms of nutrients assimilation. The assimilation is considered as the key parameter to measure the utility in the material circularity of the feed, since it reflects the use of resources and how the feeding can be aligned with the circular principles. On the other hand, the valorisation of nitrogen (NWI) through yeast cultivation was not successful leading worse results when the system is compared with the nano diet.

Regarding ZWI indicator, results are shown in Table 13. It is important to notice that zero waste indicator is referring to the capability of the system (farm operation and valorisation processes) to recover nutrients for microorganism cultivation. As yeast did not growth when waste streams from RAS6_276 were used as substrate, this indicator is not applicable. This means that that the system of both, scenarios b and c, do not have zero-waste attributes under the project perspective.

Table 13 Zero waste index of RAS6_275

	RAS6_275- Scenario a	RAS6_275- Scenario b	RAS6_275- Scenario c
INDICATORS	**RESULT**	**RESULT**	**RESULT**
Zero Waste Index (ZWI)	**0.88**	**N/A**	**N/A**

3.4 Circularity of IFN03

The goal of this trial was to evaluate the potential use of Candida meal produced within the project as novel protein source in African Catfish feed formulation. These waste streams were not tested through the valorisation experiments since IFN03 trial was running when task 1.5 was almost finished.

Therefore, some assumptions are needed to develop an approximate study of the circularity of the system. In this sense, nitrogen recovery efficiency through algae cultivation is assumed to be similar to the efficiency obtained with the valorisation of water from IFN02 as both waste streams, INF02 and IFN03 were collected under similar procedures. As Task 1.5 did not report on efficiency of nitrogen capture by yeast, a literature value is taken as an approximation (Ding et al., 2023)[9]. Information on nutrient availability is sourced from the sludge characterisation that could be done in the T1.5 and included in D1.6 just before the task finished.

Regarding the sludge collection system, faeces and uneaten feed were collected in swirl separators where settled solids gradually accumulate. Considering that the swirl separator has 63% of TSS removal efficiency, and, in accordance with the theoretical mass balance presented in Figure 3, it is estimated that that 79% of total N would be available for valorisation after the collection system.

[9] Ding H.; Li, Jiabao; Deng, F.; Huang, S.; Zhou, P.; Liu, X.; Li, Z; Li, Dong. Ammonia nitrogen recovery from biogas slurry by SCP production using Candida utilis. 2021. Journal of Environmental Management 325

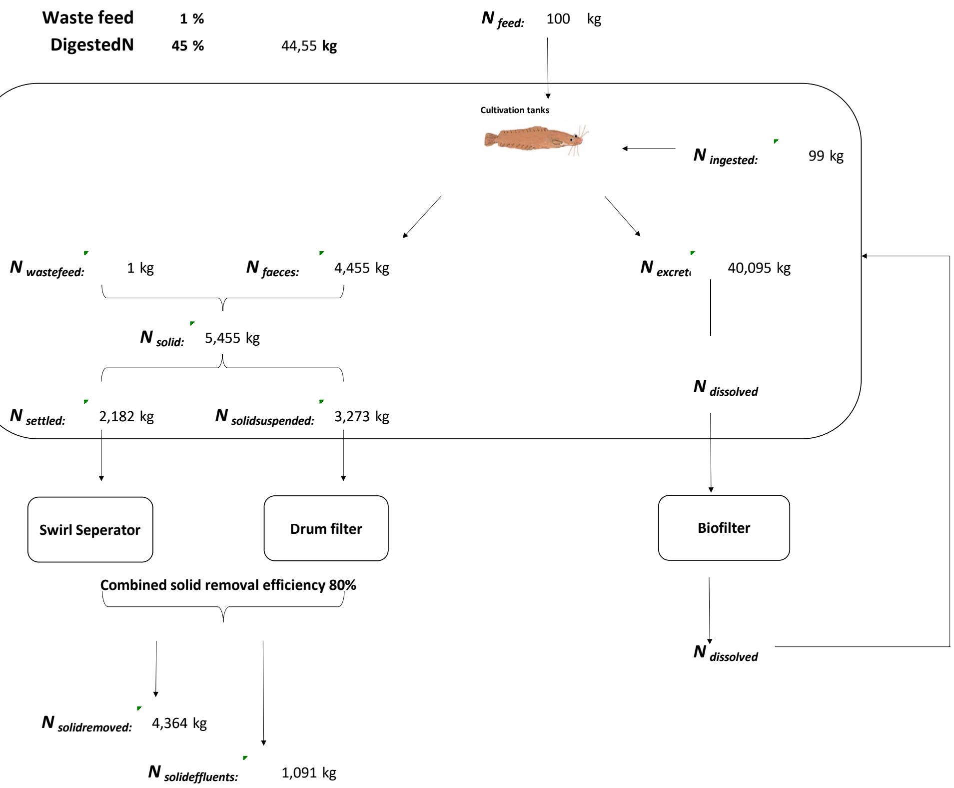


Figure 3 nitrogen mass balance for African catfish (source: ABT)

The mixture of water and solids was periodically evacuated and collected in a 5L flask. The solids were then allowed to settle and, to the extent possible, the supernatant was removed manually by siphoning. The remaining solid/water mixture was then passed through a coarse filter with a 100 µm mesh. This was followed by approximately three hours of vacuum filtration using 60µm pore size filter papers to produce concentrated sludge samples.

The circularity assessment of IFN03 aims at providing and comparing a circularity performance analysis of the valorisation of waste streams through a variety of routes. Basing on IFN02 valorisation results, and conclusions from experts in Task 1.5, yeast cultivation could be feasible with sludge from ABT but not from water. Accordingly, the following scenarios are defined:

- Scenario a) and b) different levels of Candida inclusion rates in feed (10 and 20%) and outlet water used for algae cultivation
- Scenario c) 20% of Candida inclusion and medium ingredient obtained from concentrated sludge through enzymatic process used for yeast cultivation

Next table shows the parameters used to calculate the circularity indicators. As can be noticed, the assimilation efficiency is the same irrespective of the yeast inclusion rate. The reason is that this demonstrator did not aim to study digestibility in detail, therefore assimilation information was not provided. Nitrogen assimilation efficiency is sourced from literature (Elesho, et al., 2021[10]).

[10] F.E. Elesho, S. Kröckel, D.A.H. Sutter, R. Nuraini, I.J. Chen, J.A.J. Verreth, J.W. Schrama, Effect of feeding level on the digestibility of alternative protein-rich ingredients for African catfish (*Clarias gariepinus),* Aquaculture, Volume 544, 2021, 737108, ISSN 0044-8486

Table 14 Parameters from Trial IFN03- Scenario a) (10% of candida and dirty water valorised through algae cultivation)

PARAMETERS	Data (from Trial IFN03-10%)	Data (from Trial IFN03-20%)	
Total feed provided (kg DW):	11.97	11.93	
Total nitrogen in feed (**M**) (kg DW)	0.823	0.825	
Virgin nitrogen in feed (**V**) (kg DW)*	0.745	0.660	
Eaten (ingested) feed (%)	99%	99%	
Nitrogen assimilation efficiency of new feed developed in iFishIENCi (**NE**)	87.00	87.00	
Nitrogen assimilation efficiency of conventional feed with similar properties (**NE_{av}**)	95.00	95.00	
PARAMETERS	**Data (from Trial IFN03-10%)**	**Data (from Trial IFN03-20%)**	
Nitrogen content in sludge[11] (mg/kg DW)):	34,673	40,495	
Total nitrogen sludge (kg DW) (C'_{RS})	0,0132	0,0090	
Nitrogen content in dirty water (kg DW) (C'_{RW})	5.59E-09	6.65E-09	
PARAMETERS	**Scenario a**	**Scenario b**	**Scenario c**
Nitrogen recycling efficiency for sludge (Ecs)	0%	0%	36%
Nitrogen recycling efficiency for dirty water (Ecw)	100%	100%	0%
Nitrogen recycling efficiency for algae cultivation (Ef)	56.9%	56.9%	62.5%
PARAMETERS	**Scenario a**	**Scenario b**	**Scenario c**
Nitrogen not recovered (W0) (kg DW)	0.0037	0.0025	0.0037
Nitrogen in sludge not recovered for the new value chain (Wcs) (kg DW)	0.0132	0.009	0.0084
Nitrogen in dirty water not recovered for the new value chain (Wcw) (kg DW)	0	0	5.59E-09
Nitrogen not taken by algae (WF) (kg DW)	2.41E-09	2.87E-09	1.78E-03

Once parameters are collected and interpreted, results are presented in the following tables. As expected, the linearity is better when the inclusion rate of alternative (and circular) ingredient is higher. As the utility is expected to be unaffected by the increase from 10 to 20% of yeast, the indicator MCI is more favourable for scenarios b and c. Additionally, scenario c performs best in terms of NWI indicator, as this system has a higher nutrient recycling efficiency.

[11] Total elemental Nitrogen

Table 15 Circularity assessment of IFN03 Scenario a)

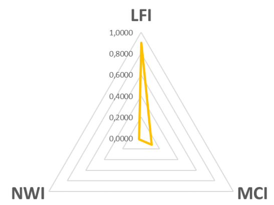
INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.90**	
Material Circular Indicator (MCI)	**0.1155**	
Nitrogen Waste Indicator (NWI)	**0.0201**	

Table 16 Circularity assessment of IFN03 Scenario b)

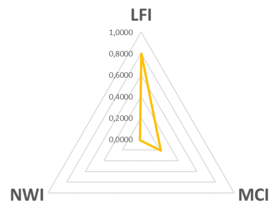
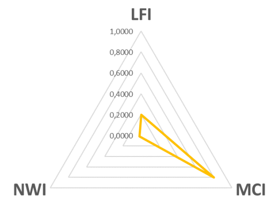
INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.80**	
Material Circular Indicator (MCI)	**0.214**	
Nitrogen Waste Indicator (NWI)	**0.0138**	

Table 17 Circularity assessment of IFN03 Scenario c)

INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.80**	
Material Circular Indicator (MCI)	**0.2138**	
Nitrogen Waste Indicator (NWI)	**0.0123**	

As can be observed in Table 19, the systems studied do not have zero waste attributes when sludge is not valorised, as sludge is the main source of nitrogen in the waste streams. Therefore, scenarios a and b only start to be considered as a zero-waste system when sludge is valorised to at least 64%. In terms of ZWI, scenario c is the most favourable since it is reflecting a high rate of nitrogen recovery. The system would be fully zero waste only if the concentrated sludge could be used directly as a substrate for the yeast, but this has been discouraged according to the observations in Task 1.5.

Table 18 Zero waste indicator of Table 19 Circularity assessment of IFN03 scenarios

	IFN03 Scenario a	IFN03 Scenario b	IFN03 Scenario c
INDICATORS	**RESULT**	**RESULT**	**RESULT**
Zero Waste Index (ZWI)	**0**	**0**	**0.214**

3.5 Circularity comparison

The developed methodology allows us interpreting all the evaluable trials and demonstrators under a same approach. As the different indicators have different target values, it may be relevant to assess them individually within the comparison.

LFI determines a good circularity performance whenever the values are closed to 0. In this sense, RAS 1_188 and RAS 5_243 have comparatively the best performance as feed had a higher inclusion rate of circular ingredients. However, in terms of circular nitrogen, RAS_5 represents the best trial. Regarding the MCI, RAS 1 and 5 are comparatively better than the rest of trials. The reason underlying this result is that, together with a lower linearity, the feed has a better assimilation in comparison with a conventional feed. When NWI is addressed, it can be observed that IFN provides better results since (evaluate with updated mass balance info)

Finally, looking at ZWI, all scenarios have zero-waste attributes except for those when the waste streams could not be used for microorganism cultivation. When both waste streams, outlet water and sludge are efficiently valorised the system is interpreted as totally zero-waste (value equal to 1), which is the cases of scenario RAS1 scenario a. Figure 4 shows graphically the circularity benchmark.

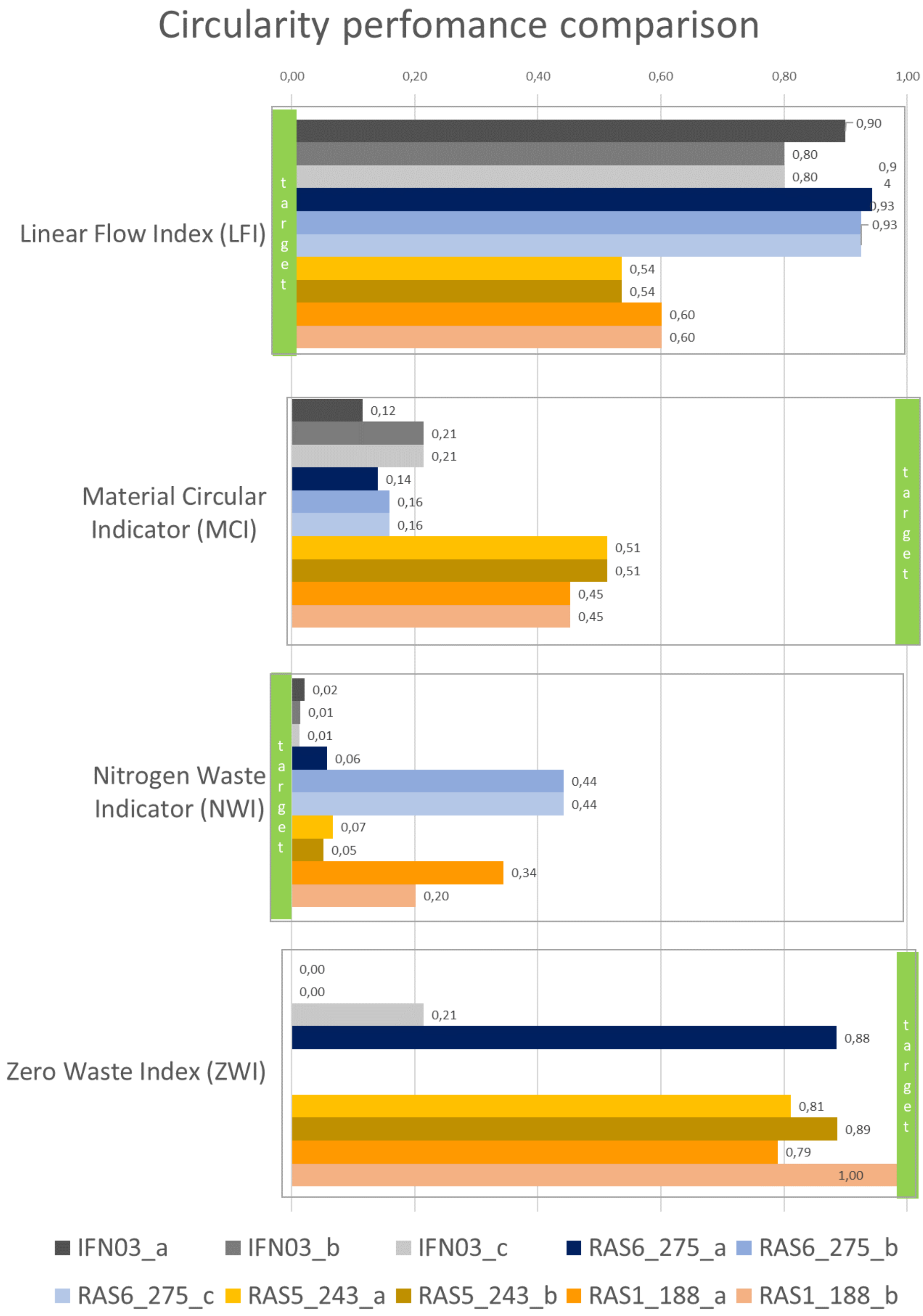


Figure 4 Circularity performance comparison

3.6 Application to open systems

The cultivation system determines the feasibility of nutrient capture and subsequent valorisation. Despite the well-known technical limitations for sludge management in open systems, this section aims at evaluating the circularity of the salmon production system where is possible to collect sludge.

To do so, the innovative technology proposed by Rang Sells for sludge capture (more details can be found in this link[12]) has been taken as a feasible alternative. It consists of a system to collect and remove waste from aquaculture net pens therefore reducing the emission of particulate nutrients to the marine environment. Although this technology is not part of the experimental work in iFIshiENCi, we consider it as a promising route for the valorisation of waste from open systems that may promote the circularity. Sludge removal efficiency of this technology has been informed by NCE seafood, which has been the basis for the circularity assessment.

To evaluate the circularity in marine cages, a theoretical scenario is described for salmon production where the nitrogen emission is modelled through the Marine fish PEFCR feed emission model v3[13]. As output from this tool, for 1 ton of conventional feed provided, 82.29 grams of nitrogen would release to the sea, of which 9.31 corresponds to particulate nitrogen. The solution from Ragn Sells could potentially collect approximately 65% of the available sludge (faeces and feed) that is produced in a traditional fish farm.

Table below summarizes the main parameters needed for the circularity assessment. They are calculated by assuming:

- 3% of algae in formulated feed
- 0.072 kg N/kg DW feed
- 80% of ingestion
- Same assimilation efficiency as obtained in above-mentioned trials feed (RAS6_275)
- 65% of solid retention efficiency
- Raw sludge (0.5-4.3% dry matter) could be reused directly as substrate for algae (without pre-treatment)
- Nitrogen capture efficiency by algae sourced from the average of the experiments in Task 1.5

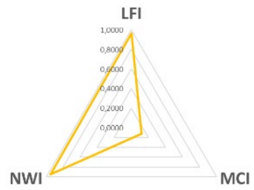
Table 20 Parameters from theoretical open scenario

PARAMETERS	**(Theoretical) open system**
Total feed provided (kg DW):	1000
Total nitrogen in feed (**M**) (kg DW)	72
Virgin nitrogen in feed (**V**) (kg DW)*	69.3
Eaten (ingested) feed (%)	80
Nitrogen assimilation efficiency of new feed developed in iFishIENCi (**NE**)	95.21
Nitrogen assimilation efficiency of conventional feed with similar properties (**NE_{av}**)	96.41
PARAMETERS	**Data**
*Total nitrogen sludge (kg DW) (**C'_{RS}**)*	9.3
*Nitrogen content in dirty water (kg DW) (**C'_{RW}**)*	64.11
PARAMETERS	**Data**
Nitrogen recycling efficiency for sludge (Ecs)	*65%*
Nitrogen recycling efficiency for dirty water (Ecw)	*N/A*

[12] For more information https://www.ragnsells.com/what-we-do/inspired/Norwegian-aquaculture/
[13] https://www.marinefishpefcr.eu/

Nitrogen recycling efficiency for algae cultivation (Ef)	*82.7%*
PARAMETERS	**Data**
Nitrogen not recovered (W0) (kg DW)	*67.3*
Nitrogen in sludge not recovered for the new value chain (Wcs) (kg DW)	*0*
Nitrogen in dirty water not recovered for the new value chain (Wcw) (kg DW)	*N/A*
Nitrogen not taken by algae (WF) (kg DW)	*1.05*

Table 21 Circularity assessment of theoretical open system

INDICATORS	RESULT	
Linear Flow Index (LFI)	**0.96**	
Material Circular Indicator (MCI)	**0.123**	
Nitrogen Waste Indicator (NWI)	**0.95**	

As part of the assessment, ZWI would not be of relevance since the theoretical scenario is calculating 100% of recoverable nitrogen therefore these nutrients would be totally available for valorisation. ZWI=1 is reflecting that no nitrogen waste would be generated through the valorisation chain, and the system would be fully "zero-waste". In this sense, open systems could have zero waste attributes when extractive species are grown together under a multi-trophic scheme (Integrate Multitrophic Aquaculture, IMTA).

4 Policy recommendations

The role that aquaculture plays within the circular economy and bio-economy is well known, since waste stream are by-products that potentially represent an input for another industrial sectors. In this context, traditional production systems such as polyculture or multi-trophic aquaculture systems already integrate principles of the circular economy.

However, during the first half period of the project, we identified that the lack of methodologies might disincentivize discourage the report on circular actions within aquaculture since there are no harmonized approaches to quantify their performance and benchmark. As well as the European Commission proposed the PEF (Product Environmental Footprint) initiative as a common way of measuring the environmental performance, harmonised circularity indicators would encourage sector to monitor and communicate circular practices aiming resource efficiency and zero waste.

Together with this, a common definition of circular aquaculture seems to be necessary within the sector. This common approach should address not only the biological and nutritional flows but also the capacity of aquaculture on facilitating the transformation of resources and waste streams into value added products.

In this context, we identified the opportunity of co-creating a set of recommendations for policy makers regarding the circularity in aquaculture by compiling comments from experts and high-level thinkers from the different fields in the sector. Under this purpose, iFishIENCi organised the "Aquaculture Going Circular" event in November 2021, where we discussed and identified ways forward in which circularity can be developed within production in a practical, efficient, and economically sound way. As a result of this event and a collaborative work with other initiatives and projects, we developed the document "Policy Recommendations for a More Circular Aquaculture" which was circulated to the main European representatives within DG MARE, JRC, RTD, ENV and AGRI. The mentioned document is available here.

Particularly, the circularity assessment task of iFishIENCi promoted the elaboration of specific recommendations regarding the definition and measurement of circularity for aquaculture practices. Based on the research and work done in the project, we recommend providing methodologies that allow assessing not only feed formulations but also, feeding and production efficiency together with potential of nutrient recovery through different valorisation routes. The methodology developed in the project covers these issues and could potentially serve as a basis for future conceptual frameworks of circular aquaculture at European level.

5 Conclusions and recommendations

iFishIENCI project has facilitated not only developing and testing new concepts for the valorisation of aquaculture waste streams but also designing an innovative approach to assess the efficiency of the proposed circular value chain. The research on different frameworks to evaluate the circularity has allowed us understanding the complexity of the aquaculture and nutrient recirculation, which require to develop an appropriated methodology to assess how well the innovative processes perform within the circular economy.

The development of a customized framework has enabled the quantification of indicators and the elaboration of recommendations for policy makers as we have been able to identify potential actions where efforts should be focused to achieve a more circular aquaculture. Providing a common definition, the research on circular ingredients, more efficient feeding systems and innovative valorisation routes are some of the key strategies that may be prioritized. The methodology can be generally applied to all feeding systems making it versatile and appropriate to provide benchmark of aquaculture production based on circular attributes.

The demonstrations carried out in the project have been evaluated by applying the indicators developed in the circularity assessment framework. The assessment addresses different scenarios for the use of raw sludge, but farms usually manage concentrated sludge so the evaluation of circularity of unconcentrated waste would only be of interest in on-site recovery solutions. Although the study has been mainly focused on the performance of RAS, where waste streams are feasibly collected, a theoretical example has been defined to evaluate the performance of a theoretical open farm with a sludge collection system. Open systems have the potential to increase circularity through the implementation of multi-trophic production systems, which allow for reduced losses of dissolved nitrogen to the environment.

Although most nutrients were already soluble and accessible for algae and yeast, the treatments to obtain medium ingredient were applied as a proof of concept to assess the potential application of the medium on algae and yeast growth, therefore a higher circularity would be expected with the direct use of unconcentrated sludge directly on the farm. On the other hand, high inclusion rate of circular ingredients in feed should be complemented with a good nutrient assimilation efficiency to allow a better performance not only in terms of linearity but also in terms of material circularity.

The valorisation experiments and circularity assessment give us the opportunity to identify new challenges to increase the circularity. From the valorisation experiments with algae, it is known that outlet water from the tested aquaculture trials contained lower nutrient concentrations than commercial medium, meaning that conditioning of the media would be necessary. This fact implies that additional nutrients which are limiting the process, such as phosphate, should be added to the waste streams which may compromise the circularity. Additionally, considering the energy intensity needed to separate microalgae from water to produce biomass for feed, it may be of relevance to evaluate further alternatives where microalgae could achieve an optimal and maximum growth using waste streams without adding extra resources. One possible alternative could be to keep the microalgae for a longer period than the water in the reactor and treating more water with the same algae. However, when optimising the microalgae production process towards highest algal biomass productivities, or towards maximum uptake of available nutrients, their respective optimal values most probably will not be at the same point, thus potentially creating a choice between circularity and production cost and revenue.

Likewise, waste streams used for yeast growth seem to be low in carbon content, which may be the reason underlying the no-growth with some waste streams. This fact may hamper the zero-waste attribute of the systems. Once again, the circularity of this value chain should be supported with additional carbon sources to guarantee the valorisation of waste. Therefore, new research areas are opened in the sense that other waste streams sources (ideally closed to the production place) should be explored to complement and increase the circularity, which is an attribute that can be now quantified through the framework developed.

The recovery of nutrients through the obtention of medium ingredient generates losses as it is not 100% efficient, but it would foster the circularity of aquaculture by transforming waste streams into suitable substrates for microorganism growth. The medium ingredient process together with the rest of valorisation routes addressed in the project are evaluated also under an environmental point of view through a Life Cycle Assessment (LCA). Once a circular process is validated, efforts should be focused on the reduction of environmental impacts. In this sense, we identify the necessity of combining both approaches, LCA and circularity, to ensure that a circular future would be also environmentally friendly.

Complementary to the evaluation of Nanno and Candida value chains, sludge from other demonstrators than those studied in this deliverable (RAS3 and IFN02) were used as a feedstock for other different types of microorganisms. Growth of the microorganisms was tested and recorded as part of WP1 but when D1.6 had been elaborated and submitted. No qualitative data were provided in terms of nutrient capture efficiency from those experiments, but the results obtained pointed out that sludge from RAS3 and IFN supported the growth of *E. glutamicum, S. warneri, E. faceum, Y. lipolytica*, *A. niger, B. licheniformis, Y. lipolytica* and *P. acidilactici*. Therefore, other potential value chains could be assessed from a circularity perspective.

To conclude, the validation and implementation of the circularity methodology allows us to confirm that the project really promotes a more circular aquaculture sector towards the implementation of practices that reduce linearity and nutrient waste, while increasing nutrient circularity and approaching zero-waste attributes.

Finally, the work done also intends to rise the interest in adopting the evaluation of circularity. As part of the results from the demo events questionnaires, we have confirmed that whenever farmers are informed on the experience of iFishIENCI they feel motivated to undertake some actions toward circular and sustainable aquaculture. In this sense, the methodology developed in the project may give them the support required to improve the aquaculture process as they can foreseeably feel encouraged to evaluate how circular those actions are.