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AQUACULTURE AND AGRICULTURE BIOMASS SIDE STREAM PROTEINS AND BIOACTIVES FOR FEED, FITNESS AND HEALTH PROMOTING NUTRITIONAL SUPPLEMENTS	
Grant agreement:	790956
Duration:	April, 2018 – December, 2022
Coordinator:	NOFIMA AS, Norway

PROJECT-BASED LEARNING

Innovative design of microbial cell factories for utilization/transformation of fish processing side-streams and production of valuable molecules

Small Research Project

'Fish processing side-streams effect on production of pigments and statins (monacolins) in fed –batch cultivation by Monascus purpureus fungus'

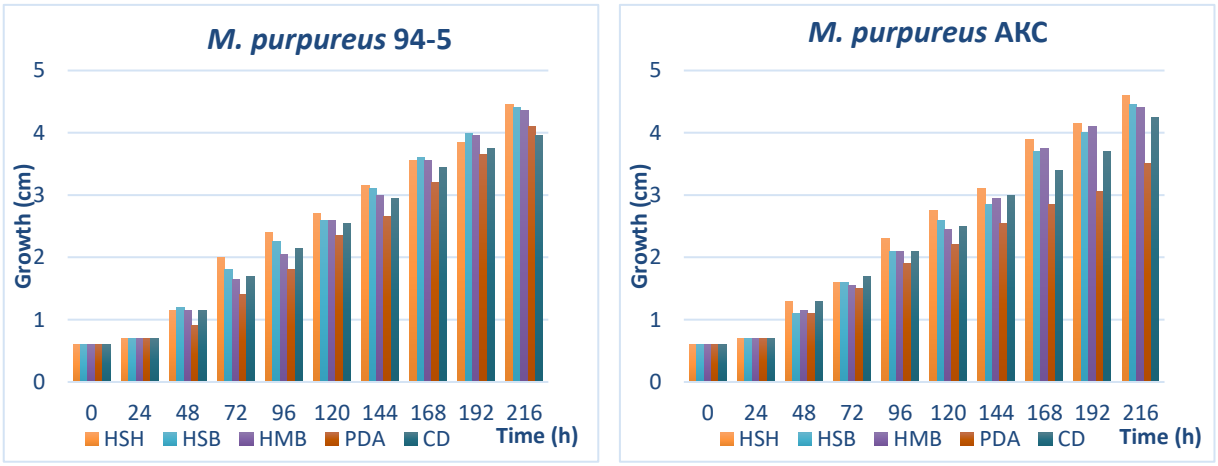
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the BBI grant agreement No 790956

<table>
<tr><td>Project title</td><td colspan="3">Fish processing side-streams effect on production of pigments and statins (monacolins) in fed –batch cultivation by Monascus purpureus fungus’</td></tr>
<tr><td>Responsible Organization(s)</td><td colspan="3">R&D Center Biointech Ltd</td></tr>
<tr><td>Project duration</td><td colspan="3">5 months</td></tr>
<tr><td rowspan="2">Research Effort Categories (%)</td><td>Basic</td><td>Applied</td><td>Developmental</td></tr>
<tr><td>10</td><td>90</td><td>-</td></tr>
<tr><td rowspan="2">Background data</td><td>Field of science</td><td>Knowledge area</td><td>Subject of investigation</td></tr>
<tr><td>Biology/Biotechnology</td><td>Microbial growth on fish side streams</td><td>Metabolic response and adaptation mechanisms</td></tr>
<tr><td>Summary</td><td colspan="3">This project focuses on appraisal of fungal growth based on media enriched with fish side streams hydrolysates. The assays are performed with Monascus purpureus fungal strains (the teleomorph 94-5 and his anamorph AKC from Sofia University collection) - producers of secondary metabolites of polyketide nature (pigments and monacolins). Screening of three fish side streams' hydrolysates for model cultures growth inhibition/stimulation was performed. The hydrolysate with the best stimulatory effect was selected. Its effect on the growth, pigment formation, and monacolin biosynthesis was evaluated in fed-batch cultures. The antioxidant capacity of the biosynthesized pigment complex was analysed. The research data indicate that the selected hydrolysate may be used as a carbon and energy source during the cultivation of M. purpureus strains for targeted biosynthesis of secondary metabolites (pigments and monacolins) with increased antioxidant capacity.</td></tr>
<tr><td>Project objectives</td><td colspan="3">Study the fungal growth on media enriched with fish side streams hydrolysates and evaluation of their capacity to promote secondary metabolites production.</td></tr>
<tr><td>Project Methods</td><td colspan="3">Along the project, the following methods and techniques will be exploited:
- Batch cultivation technique;
- Physiological and analytical control of the growth process:
o Cell viability assay;
o Soluble protein, P and N concentrations determination;
o Kinetic parameters determination;
- Pigment biosynthesis evaluation:
o Quantitative analyses
o Antioxidant capacity
- Monacolins concentration determination</td></tr>
<tr><td colspan="4">Accomplishments under project objectives</td></tr>
<tr><td>Work performance
Screening of fish by-products hydrolysates for model cultures growth inhibition/stimulation and effect on</td><td colspan="3">Results:
- <u>Microbial growth efficiency</u>: The batch cultures of M. purpureus model strains were grown on synthetic media containing three different fish hydrolysates at 1.5% concentration: Mackerel backbones, HMB; Salmon backbones, HSB; and Salmon heads, HSH. Radial growth rate (Kr) of the cultures was measured along the cultivation process. The Kr values indicated that both strains grow with the highest Kr on HSH-containing</td></tr>
</table>

secondary metabolites biosynthesis	medium: 0.0201 cm/h for *M. purpureus* 94-5 and 0.0208 cm/h for *M. purpureus* AKC. (Fig. 1). The monitoring of the morphological status and pigment biosynthesis of the model strains showed enhanced pigment formation of the nutrient media with the studied hydrolysates compared to the control one. Both the substrate and the aerial mycelium of the strains (especially of *M. purpureus* AKS) were pigmented intensely in red; excretion of a red pigment into the culture medium was observed as an intensely pigmented halo around the colony (Fig. 2 and 3). These results are consistent with the highest Kr values observed for the same cultures. The values of the kinetic parameters average growth rate (v), growth yield coefficient (Yx/s), and specific rate of substrate utilization (qs) on HSH-medium *vs.* control one are presented in Table 1. The data indicate that the model cultures grew intensively with v of 0.029 h-1 - 0.031 h-1 on the HSH medium. The *M. purpureus* 94-5 strain utilized the carbon source with good efficiency: Yx/s = 0.319 + 0.02. relatively high values for qs (0.072 + 0.01 – 0.086 + 0.02 g/g/h) were registered. These results indicate the beneficial effect of the processed side streams on the growth and development of the fungal population, especially of the teleomorph one. - <u>Effect of HSH-medium on the pigment formation and monacolin biosynthesis</u>: The dynamics of yellow pigments biosynthesis by the tested strains is shown in Figs. 4A and 5A. The extracellular yellow pigments' accumulation followed the logic of the biosynthesis of secondary metabolites - during the stationary phase, reaching values up to 3.12 AU/ml for *M. purpureus* AKC and 7.2 AU/ml for *M. purpureus* 94-5. Values within the range of 2.82 (*M. purpureus* AKC) to 11.8 AU/ml (*M. purpureus* 94-5) were registered for the red pigments (Figs. 4B and 5B). Apparently, HSH-medium has a stimulating effect on pigment formation with predominance of the red pigments (Fig. 6). The data for the HSH effect on pigment production indicate that *M. purpureus* 94-5 biosynthesizes significant amounts of yellow and red pigments under the selected culture conditions. Biosynthesis is more efficient in this strain regarding total pigment production, a confirmation of which is the calculated yield coefficients for the yellow and red pigments shown in Table 2. The strains' ability to produce statins (monacolins) was evaluated. The data presented in Table 3 show that *M. purpureus* AKS biosynthesized more monacolins when cultured on HSH-medium compared to the other model species on the same medium. The data for the antioxidant capacity of the total pigments from the two model strains (Table 3) show higher values for *M. purpureus* 94-5. These results correlate with the literature data about the general antioxidant capacity of the secondary metabolites produced by g. *Monascus* (pigments, dimeric acid, tannin, phenol, etc.) that contribute to maintaining the antioxidant status of the culture. **Conclusions:** - The physiological and biochemical investigations indicate that the growth of M. purpureus model strains on media containing 1,5% fish by-products hydrolysates is effective, and the organic substrates can be used in the innovative design of fermentation processes for the utilization/transformation of fish processing side-streams and

	production of valuable molecules. The best values for the kinetic parameters Kr, v, Yx/s and qs were obtained for *M. purpureus* 94-5 on medium enriched with HSH. - The stimulatory effect of the HSH hydrolysate on the polyketide derivatives (red pigments and monacolins) biosynthesis is due to its chemical nature – it is rich of free amino acids and small peptides that promote the red pigments biosynthesis, especially in *M. purpureus* 94-5. - The processed fish side-streams are convenient alternative carbon and energy sources for technologically feasible production of valuable products by microorganisms and represent an elegant example of circular economy in action in the fish industry.
References	1. Pirt S.J. (1975) Principles of microbe and cell cultivation, Oxford, Blackwell Scientific Publ., 4-44. 2. Miller G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. 31, 3, 426–428. 3. Taylor H. W. (1957) Formol titration: an evaluation of its various modifications. *Analysts*, 976, 488. 4. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193(1), 265-75. 5. Carlile MJ and Watkinson SC (1994) The fungi, Academic Press, London 6. Herbert P, Phipps PJ, Strange RE (1971) Chemical analyses in microbial cells. Methods Microbiol., VB, 119-175 7. Kumaran A. and Joel Karunakaran R (2007) In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Science and Technology, Volume 40, Issue 2, Pages 344-352 8. Sun J. – L., Zou X., Liu A. – Y., Xiao T. – F., (2011) Elevated yield of Monacolin K in Monascus purpureus by fungal elicitor and mutagenesis of UV and LiCl, Biol Res., 44, 377 – 382.

RESEARCH DATA:



A B

Figure 1. 6. Growth of M. purpureus 94-5 and AKC strains on solid media enriched with HMB, HSB and HSH hydrolysates.

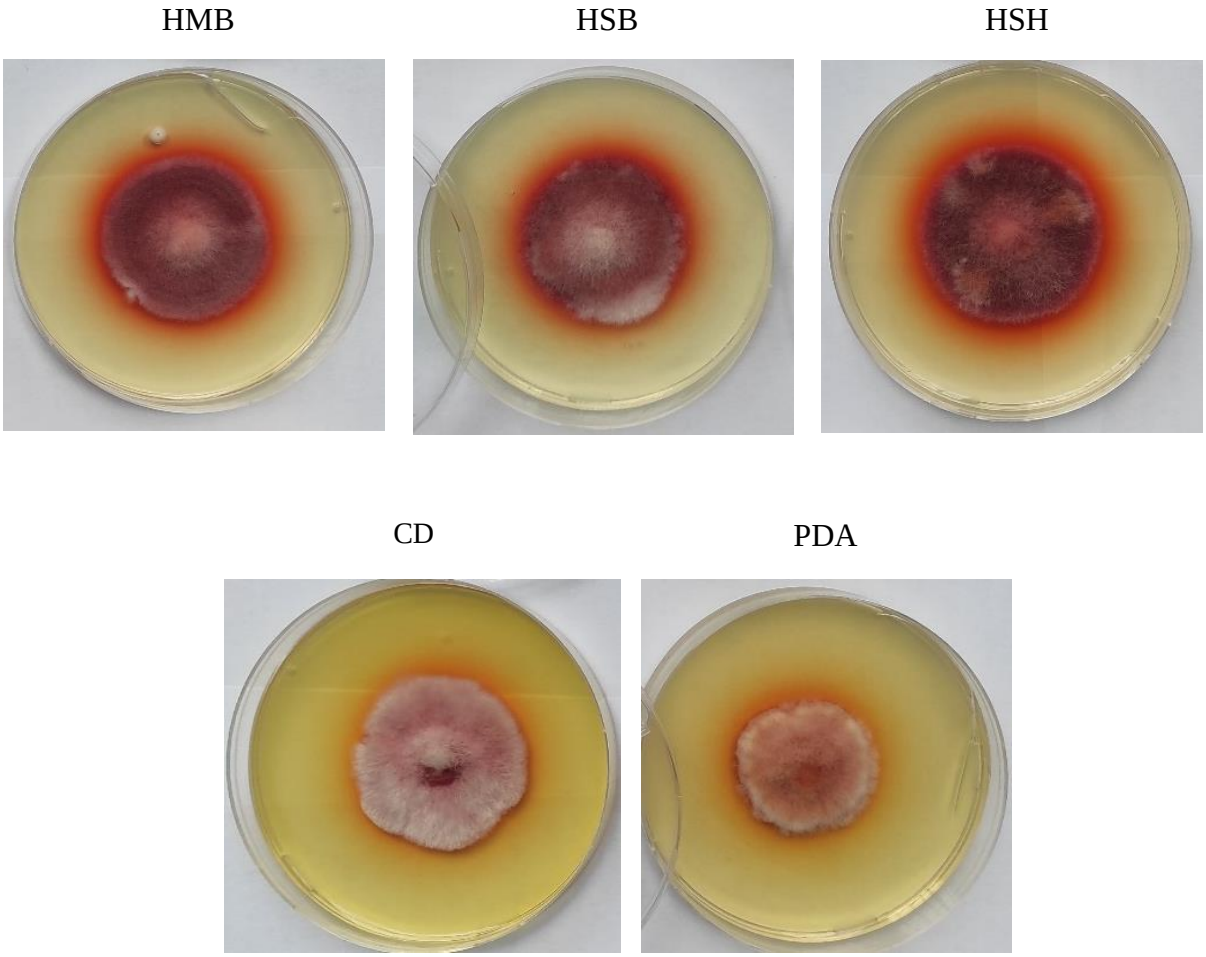
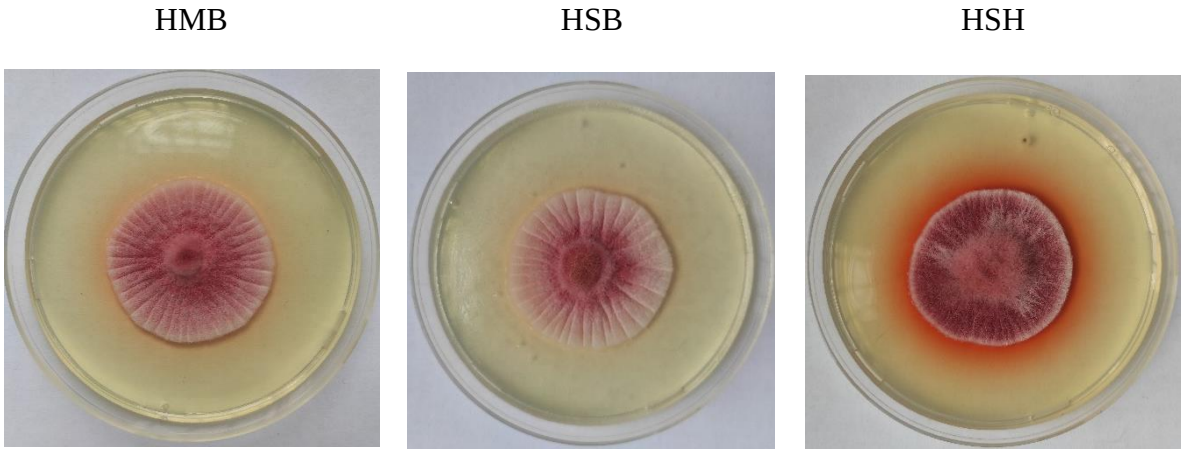


Figure 2. M. purpureus AKC grown on solid media enriched with HMB, HSB u HSH hydrolysates for 216 h at 28 °C; CD – control Capek-Dox medium and PDA -potato dextrose agar.



CD PDA

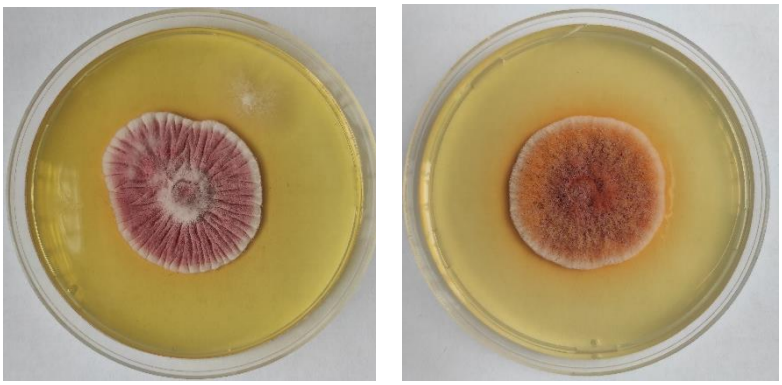
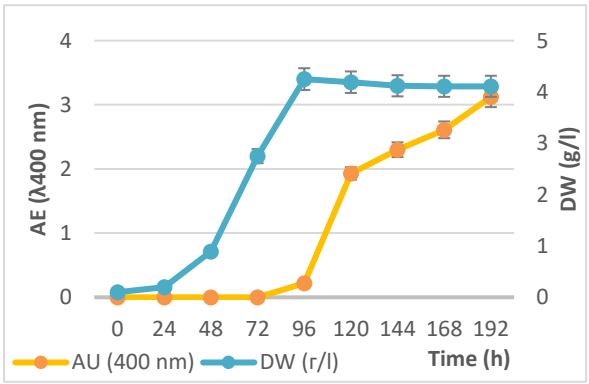
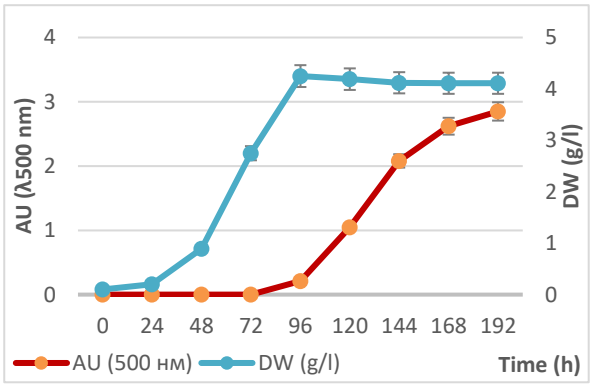


Figure 3. M. purpureus 94-5 culture grown on solid media enriched with HMB, HSB u HSH hydrolysates for 216 h at 28 °C. CD – control Capek-Dox medium and PDA -potato dextrose agar.

Table 1. Kinetic parameters of the yeast culture and inhibitory/stimulatory effect.

Parameter / Strain	Medium	V (h^{-1})	$Y_{x/c}$	q_s (g/g/h)
M. purpureus АКС	HSH	0.029	0.221	0.072
	CD	0.020	0.175	0.058
M. purpureus 94-5	ЧД-SH	0.031	0.319	0.086
	CD	0.022	0.295	0.038





A **B**

Figure 4. Biosynthesis of yellow (A) and red (B) pigments by M. purpureus АКС fed-batch culture on HSH medium.

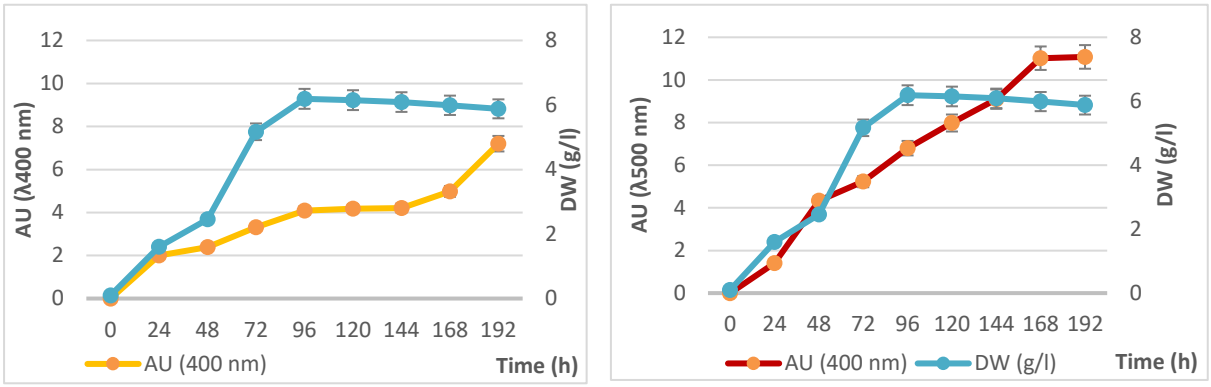
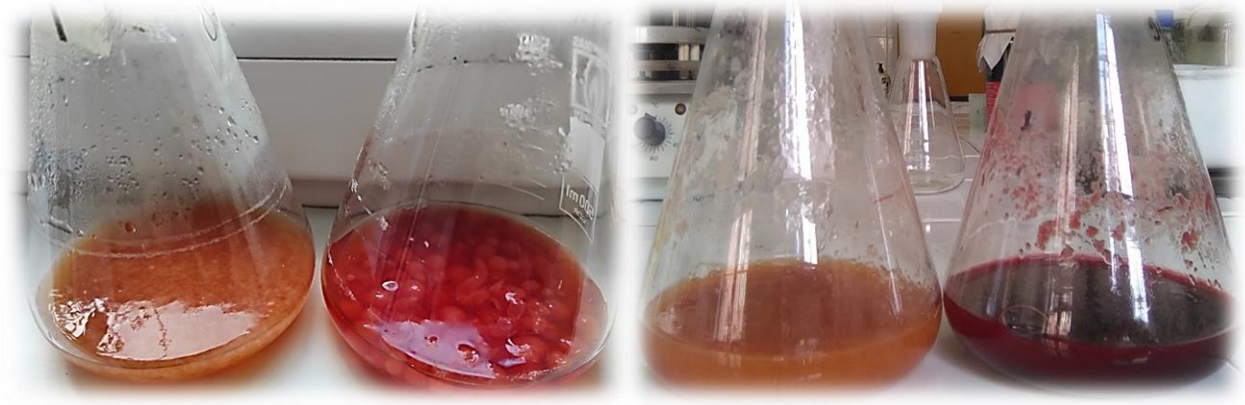


Figure 5. Biosynthesis of yellow (A) and red (B) pigments by M. purpureus 94-5 fed-batch culture on HSH medium.



A **B**

Figure 6. M. purpureus AKC (A) and 95-5 (B) fed-batch cultures after 192 h at 28 °C on HSH (right) u CD (left).

Table 2. Total pigments production (AU_{total}) and yield ($Y_{p/c}$) of *M. purpureus* AKS and 94-5 during fed-batch cultivation HSH and CD.

Parameter / Strain	Medium	λ_{400}		λ_{500}	
		AU_{total}	$Y_{p/c}$	AU_{total}	$Y_{p/c}$
M. purpureus AKC	HSH	4.06	0.224	5.71	0.315
	CD	1.47	0.085	2.25	0.131
M. purpureus 94-5	HSH	15.9	0.878	10.6	0.586
	CD	5.1	0.360	5.2	0.366

Table 3. Total antioxidant activity (AU_{total}) and mondcolis production of *M. purpureus* AKS and 94-5 during fed-batch cultivation HSH and CD.

Parameter / Strain	Medium	Monacolins (μg/ml)	Total antioxidant activity (AU_{565})
M. purpureus AKC	HSH	16.35	0.053
	CD	11.51	0.034
M. purpureus 94-5	HSH	10.07	0.363
	CD	13.32	0.041

LEARNING BENEFITS:

Learning Outcomes	After successful accomplishment of project activities, the trainees are able to:		
	Knowledge - Describe physiological and kinetic investigations with fungi - Match experimental data and theoretical considerations - Define approaches for fermentation process optimization	Skills - Apply microbiological and kinetic methods - Demonstrate techniques for microbial bioactivity assays performance fermentation process optimization - Use specialized equipment	Autonomy/responsibility - Collaborate with colleagues - Carry out tasks independently - Present and report research data
Outputs / Impact			
Target audience trained	This project is foreseen for: - Graduates and Postgraduates (MSc, PhD students) - Post-doctoral Researchers and Research Associates - Academic professionals (Tutors)		
Research results disseminated to communities of interest	Distribution of project results at intra-institutional and intra-partnership level		
Opportunities for training and professional development	The benefits for the project targets: - New specific knowledge and skills acquired - Problem/solution-based thinking mastered - Team-working abilities enhanced - Understanding of circular economy principles clarified		