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Technical report
"on the microalgae and final effluent reuse"

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Technical report on the microalgae and final effluent reuse

ACTION 6.2: Reuse

The action 6.2 aims at developing the biomass recovery systems and its possible reuse. During the monitoring campaign of the plants, we collected a sample of treat water, i.e. the inlet, inside and outlet of phytodepuration systems (see Table 1). These samples have been analysed over to test the efficiently of the plants also to evaluate the possibility of reuse of outlet water and algal biomass in according the expected results of this action (these are the expected results: *1- identification of the optimal biomass recovery system in terms of the suitable procedure for the final effluent partition between the algal biomass and the water flow; 2- analysis of the algal biomass quality data in order to select its possible reuse; 3 - analysis of the treated effluent flow quality data in order to select its possible reuse).* Specifically the analysis, that have been made to estimate of algal biomass during the monitoring activity, are total suspended solid (SST), chlorophyll a and optical microscopic observations.

DATA	pond SST [mg/l] *outlet*	photobioreactor SST [mg/l] *outlet*
04/10/2012	78.00	9
11/10/2012	74.10	16
18/10/2012	71.50	17
25/10/2012	65.00	19
08/11/2012	70.85	13
15/11/2012	65.00	12
22/11/2012	63.70	11
29/11/2012	57.20	9
05/12/2012	49.40	11
13/12/2012	52.65	18
20/12/2012	53.30	14
09/01/2013	51.35	9
16/01/2013	53.95	7
23/01/2013	54.60	11
30/01/2013	55.25	>0.5
07/02/2012	48.10	10
14/02/2013	29.25	14
21/02/2013	49.40	18
28/02/2013	42.25	12

08/03/2013	65.50	>1
15/03/2013	80.22	11
22/03/2013	85.14	14
28/03/2013	88.12	15
04/04/2013	99.15	>1
11/04/2013	88.76	30
17/04/2013	78.24	31
24/04/2013	102.15	39
03/05/2013	125.89	35
09/05/2013	132.02	45
16/05/2013	115.44	59
24/05/2013	131.56	60
30/05/2013	119.41	61
03/06/2013	128.23	66
13/06/2013	125.80	70
20/06/2013	70.03	54
27/06/2013	105.40	62
04/07/2013	108.90	66
11/07/2013	110.27	75
18/07/2013	98.72	78
25/07/2013	71.15	80
01/08/2013	94.32	85
07/08/2013	98.18	89
13/08/2013	145.20	90
21/08/2013	136.14	97
28/08/2013	150.24	90
04/09/2013	111.45	89
11/09/2013	110.38	78
18/09/2013	108.58	70
25/09/2013	100.85	66

Table 1 In this table shown all time whom we collected the samples of phytodepuration systems. Dates in bold are the times when the samples have been taken also within the plants

As the first step we tried a viable technology to recover the algal biomass. The choice of separation technology more appropriate depends on several considerations. The first element, that more conditioning concerns the nature of microalgae, which is so variable to make impossible to identify a technique best ever.

Microalgae consist microorganisms with electronegative surface charge and capable of absorbing macromolecules or extracellular material within them. Compared with other suspended particles, are presented with extremely diverse structures, giving rise to many species that differ in shape, size and mobility.
In this regard, the physical properties (size, shape), chemical characteristics (electronegativity) are the basic elements in the selection of the configuration separation. Moreover the choice of separation technology is also clearly dependent on the specific upstream of the purification process and the corresponding percentage solids input. In particular, the nature of the process, or whether this will operate in a continuous or discontinuous, can affect the opportunity of some technologies separation rather than others. Finally, the final destination of the product may require different design choices of the phase separation. On the basis of these criteria, and after a careful study of the scientific literature, the laboratory tests have only been conducted on technologies that permitted a good compromise between simple technology and economic advantage. We made tests about the membrane filtration technique, centrifugation and the simple gravimetric sedimentation. We made several testes the results are shown in the Table 2.

Technology	removal %
centrifugation	98
gravimetric sedimentation	90
membrane filtration	99

Table 2 The results of the technique using to recover the algal biomass

We decided to considerate the best results the use of the simple gravimetric sedimentation because this technique is simple, low cost and efficiently in the long time.
So in both the phytodepuration system we decide to add a sedimentation section: for the **photobioreactor** system the discharge tank act as sedimentation section because in this tank there is little movement and the algal biomass settles easily in the bottom of the discharge tank. While for the **pond** system we added a tilted plate close to the outlet of pond system that facilitated the adhesion of algal biomass and clarification of the outlet water of pond.
Therefore after we made these changes to the phytodepuration systems the algal biomass has been simpler to recover and we obtained the outlet of plants within the limits of Italian law (D.Lgs. 185/2003) to use the outlet as irrigation water or according to law D.Lgs. 152/06 we have the possibility to discharge into surface water.

As the second step the biochemical analyses have been done on the recovered biomass and on treated effluent in order to find a possible reuse.
As with any other higher plant, the chemical composition of algae is not an intrinsic constant factor, but varies from strain to strain, mainly depending on environmental parameters such as temperature, illumination, pH value and mineral content of the medium, CO_2 supply, mixing velocity, etc.
To obtain an algal biomass with a desired composition, the proportion of the different algal constituents can be modified to a certain extent by varying culture condition. But to this autochthonous algal biomass that grew in the phytodepuration plants that were placed in natural environmental, it is not possible to change the condition

of the environmental (temperature irradiation light, ecc.). In this environmental condition the chemical composition of algal biomass is shown in Table 3.
We also made the chemical analyses using elemental analysis tests (CHNOS-Varian instrument) on the recovered biomass in order to find a possible reuse.
In the synthesis the elemental analysis test process consists: before the algal biomass was dried in a water bath, properly homogenized and then introduced into a tin container in a sufficient quantity. The tin container with the sample was placed in the CHNOS instrument. The gases produced during the combustion were conveyed to a thermal conductivity detector that generated an electrical signal. This signal, processed by the Eager 300 software, provided the elemental compounds percentage.
The obtained results are shown in Table 3. The data suggest the possibility to use this autochthonous algal biomass as fertilizer for green grass (***expected results of the point 2****).*

biomass	C	H	N	S	P	K
	%	%	%	%	%	%
pond	40.52±0.43	5.70±0.08	5.39±0.12	0.20±0.01	5.21±0.25	6.49±0.11
photobioreactor	45.22±0.29	4.51±0.10	4.58±0.10	0.10±0.02	2.43±0.14	1.1±0.05

Table 3 The results of the chemical composition of the algal biomass, these are the average values on n° 13 samples

About **the expected results of the point 1** (*identification of the optimal biomass recovery system in terms of the suitable procedure for the final effluent partition between the algal biomass and the water flow*) we can say that the algal production in the two phytodepuration plants has been low during all period of monitoring activity see Table 5, but this quantity sufficient to ensure the efficient phytodepuration systems as tertiary treatment systems.
Furthermore we can said that two algal classes predominated during the monitoring activated (Action 5) have been Cyanophyceae-blue green algae (filamentous shape) and Chlorophyceae- green algae (spherical shape) (see Figure 1) and when we have these algal class in the phytodepuration system we always obtained a good efficiency in both phytodepuration plants. We could have observed, as during the summer season, the photosynthetic activity of algal biomass were greater than other season in particular we obtained values of chlorophyll a 10 time greater then values in the winter season (see Tables 4-5).

algal biomass [mg/l]	pond system	photobiorector system
autumn 2012	*98*	*26*
winter 2013	81	*19*
spring 2013	*475*	*80*
summer 2013	510	110

Table 4 The results of algal biomass concentration for the four seasons in inside plants, these are the maximum values we obtained in the plants

chlorophylla [mg/l]	pond system	photobiorector system
autumn 2012	*4.9*	*0.1*
winter 2013	2.43	*0.05*
spring 2013	*28.5*	*2.1*
summer 2013	45.9	4.5

Table 5 The results of chlorophyll a concentration for the four seasons in in inside plants, these are the maximum values we obtained in the plants

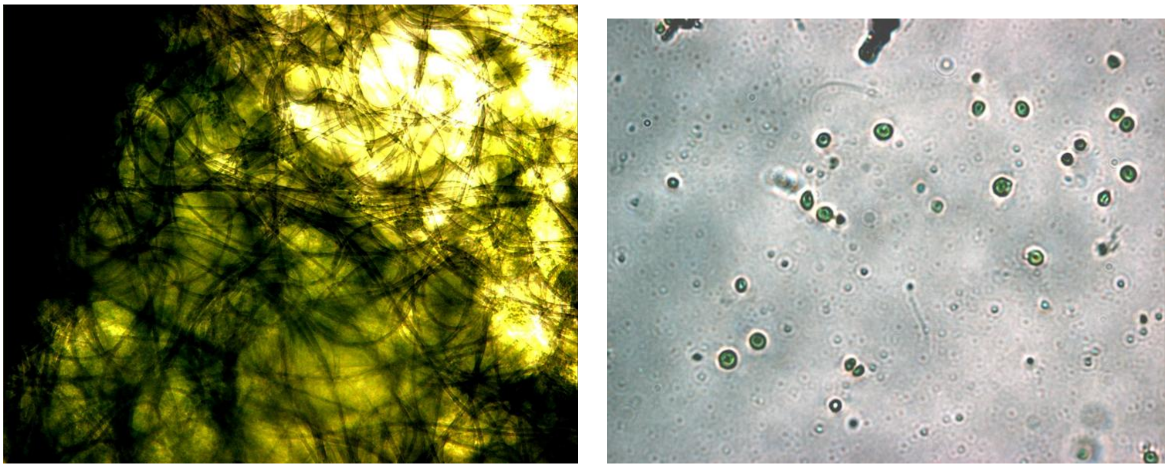

Figure 1 The two algal class present in both phytodepration plants

In the Table 6 there are the results of the quality of outlet of the phytodepuration systems (***expected results of the point 3***).
The results of the outlet (nitrogen, phosphorous, COD and SST) of phytodepuration systems show as the values of the pond system are more high than the values of the photobioreactor system, but the value of pond are always within the limit of Italian law (152/06) and moreover the values of nitrogen and of phosphorous in the outlet water make us think that we can used this outlet water to irrigate the territory close to area of the pond system enriching of nutrients. While the outlet of photobioreactor system as concentration of nitrogen and phosphorous are a lot of low so this outlet water can be used only to wet the land.

	outlet _N –NH_4 [mg/l]		outlet_P –PO_4 [mg/l]		outlet_COD [mg/l]		outlet_SST [mg/l]	
DATA	*pond*	*photobioreactor*	*pond*	*photobioreactor*	*pond*	*photobioreactor*	*pond*	*photobioreactor*
04/10/2012	8.4	2.39	3.0	0.05	86.7	20.25	78	9.0
11/10/2012	8.3	2.11	2.9	0.07	80.4	18.72	74.1	16.2
18/10/2012	16.3	2.30	2.3	0.10	73.4	16.00	71.5	17.4
25/10/2012	13.6	1.95	1.6	0.11	63.7	15.01	65	18.7
08/11/2012	6.0	1.64	1.7	0.11	70.6	11.70	70.85	13.4
15/11/2012	8.2	1.38	1.3	0.10	52.8	9.84	65	12.0
22/11/2012	5.1	1.89	1.6	0.13	74.4	15.30	63.7	10.8
29/11/2012	5.1	1.61	1.3	0.11	73.7	19.80	57.2	9.1
05/12/2012	9.7	1.16	1.4	0.04	96.8	20.84	49.4	11.0
13/12/2012	10.1	0.95	1.3	0.06	88.6	18.40	52.65	18.0
20/12/2012	11.5	0.65	1.8	0.10	90.2	14.64	53.3	13.5
09/01/2013	9.7	0.82	1.3	0.08	91.9	9.60	51.35	9.2
16/01/2013	9.4	0.99	1.7	0.06	96.8	12.15	53.95	7.4
23/01/2013	7.8	0.81	1.2	0.06	96.0	11.48	54.6	11.4
30/01/2013	14.2	0.93	1.4	0.57	96.6	14.40	55.25	>0.5
07/02/2012	9.3	0.88	0.9	0.06	100.8	7.80	48.1	10.0
14/02/2013	4.2	1.18	1.1	0.07	83.7	8.10	29.25	13.8
21/02/2013	4.1	1.42	1.3	0.07	76.4	10.56	49.4	17.5
28/02/2013	5.3	1.66	1.3	0.14	70.5	12.00	42.25	12.0
08/03/2013	4.7	1.78	1.7	0.10	72.5	15.00	45.5	>1
15/03/2013	4.1	1.95	1.9	0.11	73.2	18.00	51.35	10.6
22/03/2013	5.3	1.77	2.0	0.11	75.6	20.00	54.6	14.1
28/03/2013	6.7	1.93	1.1	0.11	53.8	19.00	42.9	15.2

04/04/2013	9.2	1.95	1.5	0.10	56.1	10.50	52.377	>1
11/04/2013	9.2	1.14	1.7	0.11	56.2	12.42	60.632	30.0
17/04/2013	9.2	1.24	1.9	0.11	61.1	14.96	71.136	31.0
24/04/2013	12.7	1.14	1.4	0.09	62.9	14.00	68.614	39.0
03/05/2013	10.3	1.21	1.6	0.07	63.2	13.92	68.263	35.0
09/05/2013	10.5	1.22	1.9	0.10	73.9	14.30	62.985	45.0
16/05/2013	9.1	0.80	1.2	0.11	79.1	12.50	70.122	59.0
24/05/2013	8.4	0.85	1.7	0.11	83.7	11.52	71.37	60.0
30/05/2013	8.5	0.74	1.4	0.09	98.7	12.60	78.936	61.0
03/06/2013	8.0	0.70	1.3	0.12	87.6	22.50	78.39	66.0
13/06/2013	14.2	0.36	1.8	0.08	83.7	19.88	69.888	70.0
20/06/2013	18.2	0.54	1.6	0.07	71.7	18.63	78.26	54.0
27/06/2013	14.0	0.67	1.9	0.08	75.3	21.17	75.816	62.0
04/07/2013	7.1	0.48	1.2	0.09	71.1	16.43	72.54	66.0
11/07/2013	6.0	0.50	1.5	0.09	67.6	20.65	79.261	75.0
18/07/2013	4.4	0.57	1.4	0.09	72.8	15.00	81.627	78.0
25/07/2013	5.0	0.58	1.0	0.05	73.0	14.21	76.57	80.0
01/08/2013	6.4	0.71	1.4	0.06	80.0	14.40	69.888	85.0
07/08/2013	2.2	1.03	1.2	0.07	75.3	15.04	74.048	89.0
13/08/2013	2.6	0.66	1.0	0.06	72.9	12.30	73.71	90.0
21/08/2013	2.7	0.75	1.3	0.07	73.8	15.50	78.936	97.0
28/08/2013	3.4	0.79	1.0	0.07	73.6	14.79	72.54	90.0
04/09/2013	1.9	0.53	1.2	0.07	60.5	15.00	72.098	89.0
11/09/2013	1.9	0.48	1.4	0.07	59.2	17.16	67.925	78.0
18/09/2013	1.4	0.38	1.3	0.07	57.7	16.96	83.616	70.0

25/09/2013	5.7	0.44	1.2	0.06	62.0	16.80	73.71	66.0

Table 6 The outlet results of the phytodepuration plan