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NITROGEN AND *Escherichia coli* REMOVAL IN FACULTATIVE FINISHING LAGOONS RECEIVING TREATED URBAN WASTEWATER

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Abstract

The scarcity of water resources, especially in the agricultural sector, is an increasing problem both in developing and industrialized countries. Recent studies and reports have shown the high potential of wastewater reuse mostly in South European countries. In this paper, we study the possibility to reuse wastewater from the Santerno full-scale wastewater treatment plant located in Imola (Bologna, Italy). Specific monitoring campaigns have been carried out in Basin 1 of the natural finishing treatment of the plant and these data are analysed and discussed. The Nitrogen and *Escherichia coli* degradation has been analysed with respect to the nitrification/denitrification and disinfection processes in the water volume. Furthermore, we have implemented a prediction model for the *Escherichia coli* degradation in Basin 1 and compared the results with the measured data. The comparison results are encouraging, showing that a future implementation of the model on Basin 1 is possible. Finally, the first data collected on a pilot plant designed and realized near Basin 1, are discussed. The *Escherichia coli* data collected in pilot plant and Basin 1, show that the main part of the disinfection process occurs in the upper layer of Basin 1 (around 60 cm). Consequently, this layer is crucial in order to define future management policies that can be tested on the pilot plant and then adopted on the full-scale plant.

Keywords: disinfection; finishing lagoon; nitrogen removal; solar radiation; wastewater reuse

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1. Introduction

The lack of water resources availability for human activities is a very critical and current problem all over the world. This problem is decisive in agricultural sector where the water consumption is very high referred to the annual global freshwater withdrawals (Tran et al., 2016). In this context water reuse has a key role. In particular, the developing of smart wastewater reuse practises can be a very interesting future solution. The global wastewater reuse volume is increasing and this trend is expected to be confirmed in the coming years (Kirhensteine et al., 2016) (BIO by Deloitte, 2015)(Sanz and Bernd, 2014).

Therefore, the rising attention on wastewater reclamation pushed on the development of guidelines and regulations both for the promotion of wastewater reuse and for human health and environment protection, by defining correctly the water quality indicators, their specific reuse and threshold values.

At European level, no specific directive for wastewater reuse has been implemented yet. Instead, several environmental directives used at member states and regional levels exist for the implementation of national laws and standards. Regulations are thus highly heterogeneous, especially in terms of intended uses, analytical parameters and permitted threshold values. This regulation heterogeneity represents one of the main barriers to the development of wastewater reuse at European level. Recently, the European Commission (EC) puts water reuse as key point of the Circular Economy action plan to overcome this problem. In 2016 the EC asked to the Joint Research

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Centre (JRC) to develop a technical report for water reuse in agricultural irrigation. After a first draft in October 2016, the JRC published a final version in June 2017 (Alcalde-Sanz and Gawlik, 2017) in which minimum quality requirements for water reuse have been proposed dividing the reclaimed water in four quality classes associated to different agricultural uses. Moreover, the EC requested an additional study to the Technical University of Munich who published a complete report in October 2017 (Drewes et al., 2017). Starting from the studies, on May 2018 the European Commission published a "Proposal for a Regulation Of The European Parliament And Of The Council on minimum requirements for water reuse" in order to overcome this regulation heterogeneity.

Table 1 shows the legal thresholds values for irrigation reuse in five European countries: Italy (DM 185, 2003), Spain (RD 1620, 2007), France (JORF 153, 2014), Greece (CMD 145116, 2011), Cyprus (Law 106, 2002) compared to the 2018 EC proposal (European Commission, 2018). The legal thresholds are referred to five main parameters: Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Nitrogen (TN), Ammonium (NH_4^+) and *Escherichia coli (E. coli)*. The development of wastewater reuse for irrigation deals with several barriers: cultural, economic, food security. Moreover, most of the existing Wastewater Treatment Plants (WWTPs) have been designed and built to respect the limits for discharge in natural water bodies. Thus, their adaptation for irrigation reuse will be an important challenge in the near future. This adaptation can be complicated in large plants due to the lack of large surface availability near them. Indeed, wastewater reuse for irrigation needs large surfaces for storage basins. In medium plants, less than 100000 Population Equivalent (PE), this availability appears more feasible even if the existing processes are verified and studied with respect to two main requirements: 1) to respect the legal thresholds and, 2) to guarantee the water storage in order to comply with irrigation needs.

Thus, the adaptation of existing WWTPs to irrigation reuse needs appears to be a very interesting option even if its feasibility is also connected to the achievement of very stringent legal thresholds, specifically *E. coli* (Table 1). On the other hand, the achievement of COD and TN limits could be easier as they are similar with the ones for discharge in water bodies.

Nevertheless, Nitrogen discharge in soils must be taken into account because high concentrations can reduce the crops quality due to overstimulation, lodging or maturity delay (Lazarova and Bahri, 2005). Indeed, Nitrogen compounds could be already present in the soil due to addition as fertilizer. Besides, a reduction of Nitrogen concentration enables discharging effluent wastewater after irrigation in water bodies, with a high tendency for eutrophication phenomena (Mancini, 2004).

In this context, we studied the existing WWTP of the city of Imola (Bologna, Italy), called "Santerno municipal WWTP", where a traditional municipal plant with predenitrification/nitrification is followed by five facultative lagoons as tertiary finishing treatment. The final aim of the study is to adapt the existing plant for irrigation reuse diverting a part or the entire effluent flow from the first facultative lagoon (Basin 1) fulfilling the seasonal irrigation requirements. This adaptation can be implemented only if the effluent from Basin 1 will respect the legal thresholds. We refer on the Italian thresholds (DM 185, 2003) as they are very stringent, in some cases (*E. coli*) more stringent than the ones proposed by EU countries (Table 1).

In wider terms, the implementation of natural wastewater treatment systems allows for Nitrogen reduction, thanks to two main processes due to algal and biomass activities (Malschi et al., 2018), as well as for natural disinfection that depends on the solar irradiation, pH and temperature and takes place both in aerobic and anoxic conditions (Liu et al., 2016; Pozo-Morales et al., 2014).

We studied more particularly the nitrification/denitrification and natural disinfection processes occurring in Basin 1 through the analysis of the data collected during specific monitoring campaigns conducted from May 2016 to July 2017. Furthermore, we have implemented the *E. coli* degradation model based on the dispersed flow equation from Wehner and Wilhelm (Wehner and Wilhelm, 1956) in order to test its usability in full scale case. We studied the disinfection capacity of Basin1 with respect to solar irradiation variations both on the water surface and in the water column.

Table 1. BOD, COD, TN, NH_4^+ and *E. coli* legal limits for irrigation reuse in five European countries and EU Proposal in 2018

Parameter	***Italy*** (DM 185, 2003)	***Spain*** (RD 1620, 2007)	***France*** (JORF 153, 2014)	***Greece*** (CMD 145116, 2011)	***Cyprus*** (Law 106, 2002)	***EC proposal*** (European Commision, 2018)
BOD (mg/L)	20	-	-	10-25	10-70	10-25
COD (mg/L)	100	-	60	-	70	-
TN (mgN/L)	15	10	-	30	15	-
NH_4^+ ($mgNH_4^+/L$)	2	-	-	-	-	-
***E. coli* (CFU/100mL)**	10[a]	0-10^4	250-10^5	5-200	5-10^3	10-10^4

a. It is the limit for 80% of the samples while 100 CFU/100mL is the maximum limit for all cases. The limit is higher using natural systems (phyto-depuration or lagoons) becoming: 50 for 80% of the samples while 200 CFU/100mL is the maximum limit for all cases.

In particular, the presence of the aquatic macrophyte *Lemna minor (Lemna)* on the water surface has been estimated during the monitoring campaigns and taken into account in the model implementation as it strongly influences the light penetration in the water column.

Finally, a pilot plant has been designed and realised in the Santerno plant area in order to study the natural disinfection process in the upper layer of Basin 1 measuring the *E. coli* concentration.

The study has been developed in the frame of a partnership project between the Department of Civil, Chemical, Environmental and Materials Engineering (DICAM) of the University of Bologna and the multiutility HERA S.P.A., responsible of the water and wastewater management in Bologna region. The general aim of the project was the management optimization of the Santerno plant with the specific aim of the implementation of the lagoon basins for irrigation reuse by analyzing, for the first time in this site, chemical and microbiological phenomena.

2. Materials and method

The study is based on data collected during measurement campaigns conducted from 25 May 2016 to 24 July 2017 both in the first lagoon (Basin 1) of the tertiary natural treatment phase of the Santerno full scale WWTP and in the pilot plant located in the plant area. The full-scale plant is fed on urban wastewater from the city of Imola and hinterland, 75000 PE, with an average influent flow rate of 25000 m^3/day. As shown in Fig. 1, the overall plant scheme can be divided in two main parts: primary/secondary treatments and natural finishing treatments. After the primary treatment (screening) without primary sedimentation, the influent sewage goes to the secondary treatments made by denitrification and nitrification tanks as active sludge process and secondary sedimentation. Finally, five natural treatment basins provide for the finishing and natural disinfection treatment before the final discharge into the Santerno river.

Basin 1 follows the secondary treatments by treating half the effluent flow that can easily be by-passed for irrigation reuse (see dotted arrow in Fig. 1). Its volume is around 23000 m^3 with a water surface of 14000 m^2, consequently its Hydraulic Retention Time (HRT) is around 2 days. Besides, *Lemna minor* grows in this basin occupying its whole surface during summer and leading to an equilibrium between phyto-treatment and Free Water Surface (FWS) lagoon.

In a first step of the study, we divided the basin into four sections perpendicular to the main flow direction. Hence, we measured the water depth at each black point for each section obtaining the cross-sections profiles (Fig. 2). Afterwards, we measured Temperature (t) and Dissolved Oxygen (DO) along the water column at each point marked with black/white dots in Fig. 2, using the multiparameter system YSI 556. Moreover, we collected samples in the middle of the water column of the same points in order to measure Ammonium Nitrogen (NH_4^+-N), Nitrate Nitrogen (NO_3^--N), Total Nitrogen (TN) and *E. coli*. Ammonium Nitrogen has been measured with the Ion Selective Electrod Crison 9663C, NO_3^--N with Ion Chromatograph DX-120 and Total Nitrogen according to the APHA methods for water and wastewater (APHA, 1998).

E. coli were enumerated by membrane filtration method using membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC) according to the EPA Method 1603 (Usepa, 2009). After filtration, using a Whatman system, the filters were placed on the mTEC in Petri plats and incubated at 35°C for 2 hours and, afterwards, at 44.5°C for 22 hours.

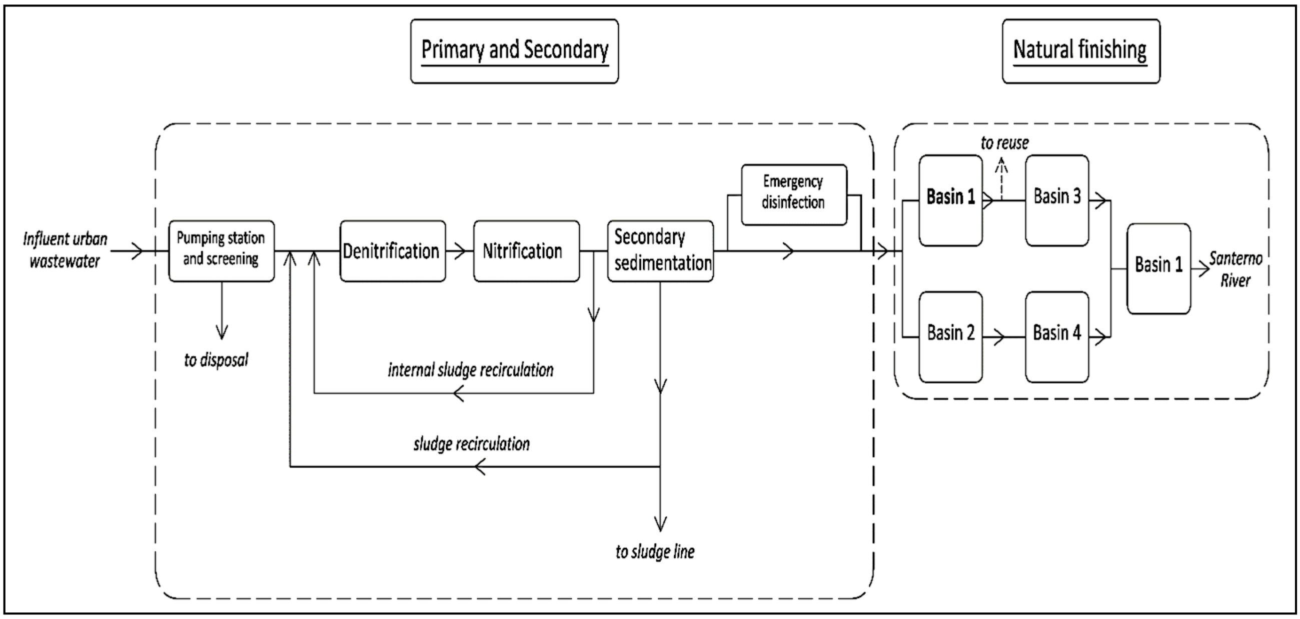

Fig. 1. Treatment scheme of the Santerno WWTP in Imola (Bologna, Italy)

We implemented a model for *E. coli* degradation in Basin 1 using the measured input data. We then compared the simulated results with the data measured in section D-D'. The model is based on the most common solution for the steady-state differential equation (Eq. 1) in its dimensionless form (Wehner and Wilhelm, 1956). The Equation is commonly used for chemical reactors design. In 1969 Thirumurthi proposed its application to BOD removal modelling (Crites et al., 2006) in facultative ponds under the hypothesis that they can be considered something between Plug Flow Reactor (PFR) and Completely Mixed Flow Reactor. Recently, the equation has been adapted to *Fecal Coliform* and *E. coli* degradations in constructed wetlands by Khatiwada N.R and Polprasert C. (Hamaamin et al., 2014; Khatiwada and Polprasert, 1999) and under the same hypotheses. Consequently, we implemented the dispersed flow equation under the following hypotheses:

1) hydraulic behaviour between Plug Flow Reactor and Complete Mixed Flow Reactor;

2) the mechanism of pathogen removal is due to the effects of temperature, solar radiation, sedimentation, adsorption and filtration.

$$\frac{C_e}{C_0} = \frac{4a_1 e^{\frac{1}{2d}}}{(1+a)^2 e^{\frac{1}{2d}} - (1-a)^2 e^{\frac{1}{2d}}} \qquad (1)$$

where: Ce = influent *E. coli* (CFU/100 mL); C_0 = effluent *E. coli* (CFU/100 mL); $a = \sqrt{1+4kTd}$ (-); $k = K_t + K_f + K_i$ overall removal rate coefficient (day^{-1}); $K_t = K_{t,20} * \Phi^{t-20}$ removal rate coefficient due to temperature at °C (day^{-1}); $K_i = \varphi * I_{av}$ removal rate coefficient due to solar radiation (day^{-1}); $I_{av} = \frac{I_0}{\tau \cdot h}(1-e^{-\tau h})$ average solar radiation (cal/m^{2}day); $K_f = \frac{4}{\pi}\eta\alpha\frac{u(1-\vartheta)}{d_c}$ removal rate coefficient due to adsorption, filtration and sedimentation (day^{-1}); $\eta = 0.9 A_S^{1/3} \cdot \left(\frac{K_B T_a}{u d_c d_p u}\right)^{2/3} + \frac{2}{3} A_S \left(\frac{d_p}{d_c}\right)^2 + \frac{(\rho_P - \rho) g d_p^2}{18 \mu u}$ removal efficiency (-); $A_S = \frac{2(1-\in^5)}{2-3\in+3\in^5-e\in^6}$ parameter accounting for the effect on adjacent media grains on the flow about a collector (-); $\in = (1-\theta)^{1/3}$ parameter accounting for the porosity (-).

Table 2 shows the parameter values used in the model implementation. They are based on literature review and a previous study of the authors (Fiorentino et al., 2016). In this case, a specific measurement campaign on the solar irradiation (I_0) has been carried out in the Santerno WWTP site. The solar irradiation was measured with a PIRSC-GEOVES pyranometer located near Basin 1 and the data were acquired and stored with a data logger stand-alone, dataTaker DT 80, at a frequency of one per minute.

In order to implement the model to Basin 1 we have followed different steps. First, the flow velocity in each chosen section has been calculated with Chézy formula, considering the influent flow rate and the cross area measured. Second, Basin 1 has been schematized as four rectangles, where flow velocity (v) and water depth (h) remain constant (Fig. 3). Third, Basin 1 has been discretized into 10m spaced sections and calculated the corresponding HRT. Finally, (Eq. 1) has been solved assigning the *E. coli* input concentration as C_0 in the first section. The natural disinfection process mainly depends on the solar radiation and its capacity to pierce through the water.

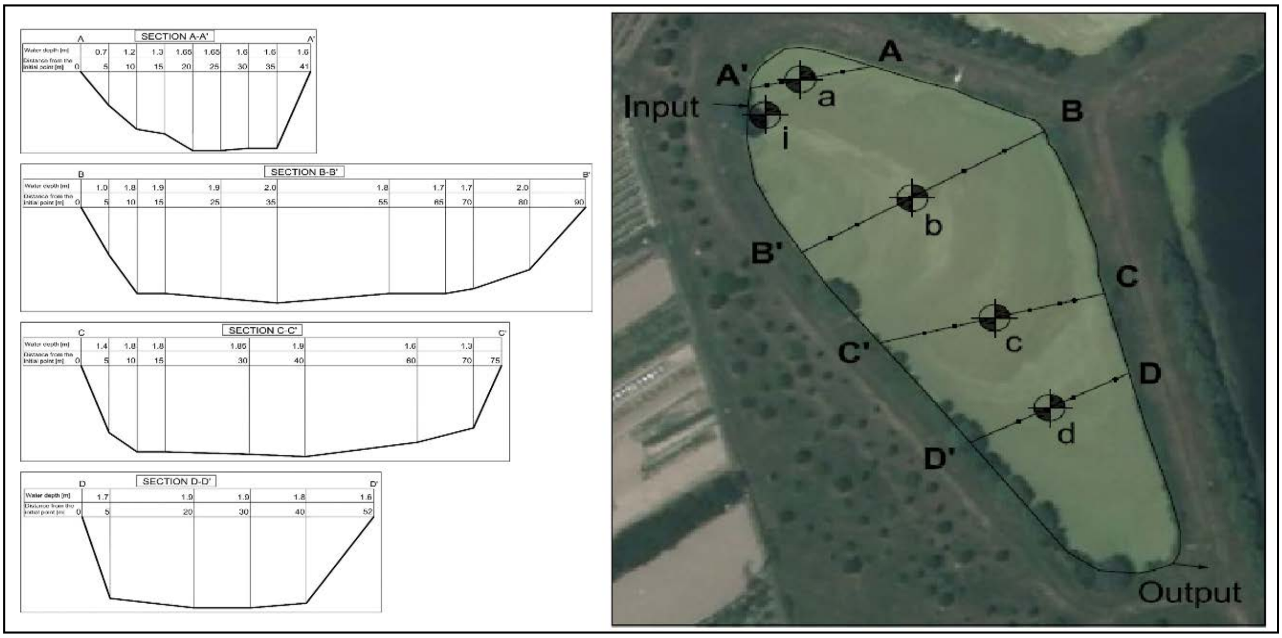

Fig. 2. Basin 1: cross-sections profiles (left) and aerial view (right) with sampling points (i, a, b, c, d) marked with black/white dots and water depth measurement points marked with black dots

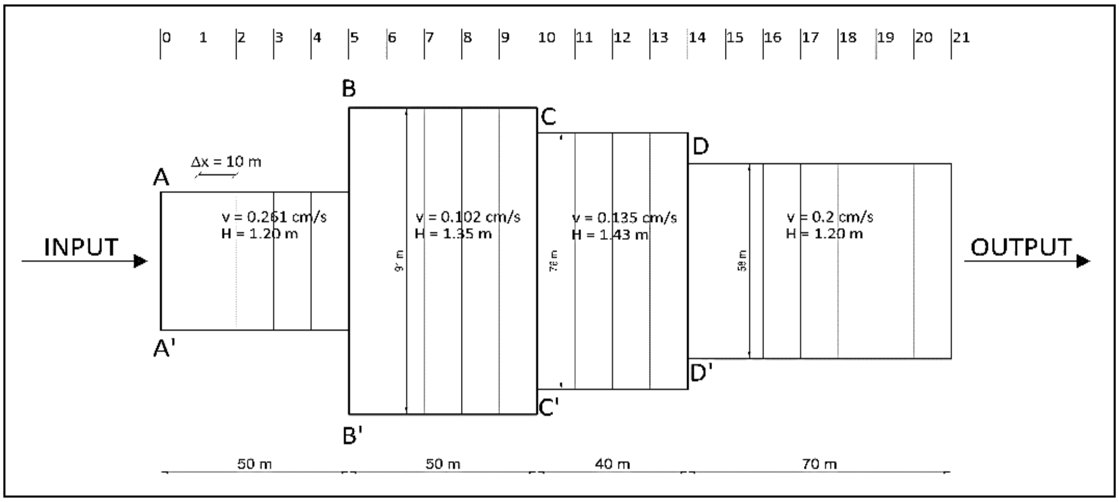

Fig. 3. Basin 1: scheme for *E. coli* model implementation

It is possible that the solar radiation pierces only through the upper layer of Basin 1 so the disinfection effect should not interest the entire water column. Moreover, the presence of *Lemna* on the water surface shades the solar radiation reducing this capacity and its thickness intensify the effect. Starting from these hypotheses, we first verified the *E. coli* degradation in the pilot plant realized in the Santerno WWTP area, by collecting samples in two points: near the input (PP_input) and output (PP_output). These sampling points are marked with black/white dots in Fig. 4.

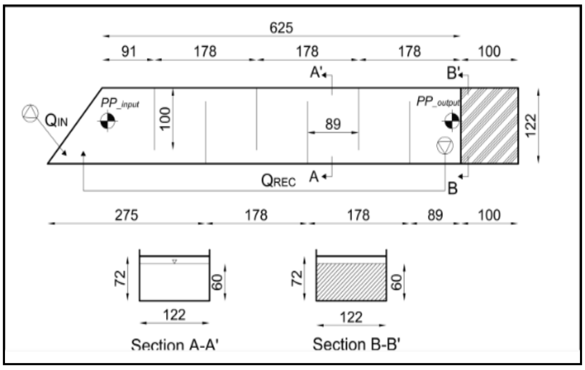

Fig. 4. Pilot plant: plan and sections (left) and picture from the outlet (right). Measures are in centimetres and the black and white dots represent the sampling points

The pilot plant has been designed to be a plug flow reactor and to represent the upper layer of Basin 1 (around 60 cm). The tank is equipped with six baffle walls and two pumps: the first for inlet flow (Q_{IN}) and the second for recirculation flow (Q_{REC}). The input and recirculation flow rates have been fixed to 0.02 l/s and 1.15 l/s in order to have HRT equal to 2 days. The water surface was covered by a constant thickness of *Lemna minor* during all the experimental period.

3. Results and discussion

3.1. Nitrification/Denitrification processes on Basin 1

We present the results for the Ammonium Nitrogen, Nitrate Nitrogen and Total Nitrogen analysis in Table 3. The basin surface covered by *Lemna* has been evaluated in each measurement campaign to take into account its finishing effect (Fig. 5). Observing the NH_4^+-N data in section A-A', three periods can be identified:

1) from 25 May 2016 to 26 October 2016 the NH_4^+-N concentration is in the range 3.5 - 8.6 mgN/L;

2) from 30 November 2016 to 24 May 2017 with lower values (1.3 - 1.7 mgN/L).

3) the last NH_4^+-N value in section A-A' return in the range of the first period (6.0 mgN/L).

Table 2. *Escherichia coli* degradation model: parameters used

Parameters		***Unit***	***Value***	***Reference***
d	Dispersion number	(-)	0.15	(Polprasert et al., 1998)
$K_{t,20}$	Removal rate coefficient at 20°C	(day^{-1})	0.047	(Khatiwada and Polprasert, 1999)
ϕ	Temperature coefficient	(-)	1.07	(Mancini, 1978)
φ	Light mortality constant	(cm^2/cal)	0.0103	(Sarikaya et al., 1987)
τ	Vertical light extinction coefficient	(m^{-1})	25 (with *Lemna*) 1 (without *Lemna*)	(Khatiwada and Polprasert, 1999)
α	Sticking efficiency	(-)	0.003	(Khatiwada and Polprasert, 1999)
θ	Porosity	(-)	0.52	(Khatiwada and Polprasert, 1999)
d_c	Duckweed root diameter	(m)	1.76×10^{-4}	(Cedergreen and Madsen, 2002)
d_p	*E. coli* diameter	(m)	1×10^{-6}	(Khatiwada and Polprasert, 1999)
ρ_p	*E. coli* density	(kg·m^{-3})	1050	(Khatiwada and Polprasert, 1999)

These variations are due to different management policies in the Primary/Secondary treatments, especially for the denitrification/nitrification processes. As the aim of this study is to investigate the possibility to reuse the wastewater coming from a common and existing urban WWTP, the capacity of the natural treatment basin to face such variations is crucial to respect the legal thresholds.

The 25 May 2016 data show ammonium nitrogen decrease while nitrate nitrogen increases and TN remains under the Italian legal thresholds for irrigation reuse. Indeed, the input data are typical of a partial nitrification of the secondary treatment. The ammonium nitrogen reduction in the basin is only due to the nitrification process and there is no evidence of photosynthetic activity. Moreover, *Lemna* did not influence the process as it occupied only a small area near the inlet section.

On 15 June 2016 the conditions started to change with a decrease of NO_3^--N in section D-D' as well as a lower NH_4^+-N removal efficiency even if NH_4^+-N in input is comparable with the previous case.

In this case, the data reveal typical aerobic lagoon conditions with photosynthetic activity. The finishing effect of *Lemna* started to influence the process because it covered approximatively one third of the basin surface near the bank.

On 13 July 2016 almost all the basin surface was covered by *Lemna*. We observe a maximum finishing effect as shown by the TN removal efficiency, around 40%. In particular, the highest TN decrease is observable in the middle of the basin, at sections B-B' and C-C, where *Lemna* is better established (Fig. 5). The TN removal efficiency increases on 26 October 2016 due to lower nitrification effect, while NH_4^+-N reduction is minimal. Moreover, *Lemna* covers one fourth of the surface near the D-D' section, but its finishing effect is again minimal because its main part is not in vegetative phase. On 30 November 2016 and 22 February 2017, the finishing effect of the lagoon is much lower than before. Indeed, these analyses were conducted during the winter season when the photosynthetic activity was almost absent and there was not *Lemna* on the surface.

Table 3. Basin 1: Ammonium Nitrogen (NH_4^+-N), Nitrate Nitrogen (NO_3^--N) and Total Nitrogen (TN) data in the middle of the sections A-A', B-B', C-C' D-D'

Meas. campaign	***Sections***													
	A-A'			***B-B'***			***C-C'***			***D-D'***			***Removal efficiency***	
	NH_4^+-N	***NO_3^--N***	***TN***	***NH_4^+-N***	***NO_3^--N***	***TN***	***NH_4^+-N***	***NO_3^--N***	***TN***	***NH_4^+-N***	***NO_3^--N***	***TN***	***NH_4^+-N***	***TN***
	(mgN/L)	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(mgN/L)*	*(%)*	*(%)*
25 May 2016	8.6	7.5	16.2	3.5	8.9	18	3.9	9.2	13.3	3.7	9.3	13.2	57	19
15 June 2016	6.7	6.8	13.7	4.2	7.3	14.5	4.4	7.3	11.8	3.7	7.2	10.9	45	20
13 July 2016	3.2	5.2	8.6	3.1	3.2	6.5	2.7	2.6	5.4	2.6	2.6	5.2	19	40
26 October 2016	3.5	7.9	11.9	3.5	8.3	12	3.2	8.6	11.8	3.2	8.5	11.8	9	1
30 November 2016	1.3	8.9	11.3	0.9	11.1	12.1	1.0	11.0	12.0	1.0	10.0	11.2	23	-
22 February 2017	1.4	20.1	21.9	1.4	19.2	22.0	1.4	18.7	20.1	1.6	18.7	21.0	-	4
22 March 2017	1.7	12.8	14.5	2.2	11.3	13.7	2.4	10.9	13.4	2.4	10.8	13.2	-	9
24 May 2017	1.7	12.9	14.8	1.6	14.3	16.0	1.5	13.8	15.3	1.5	14.6	16.4	12	-
21June 2017	6.0	4.4	10.3	5.3	4.7	10.2	4.4	4.3	8.8	4.4	4.8	9.3	26	10

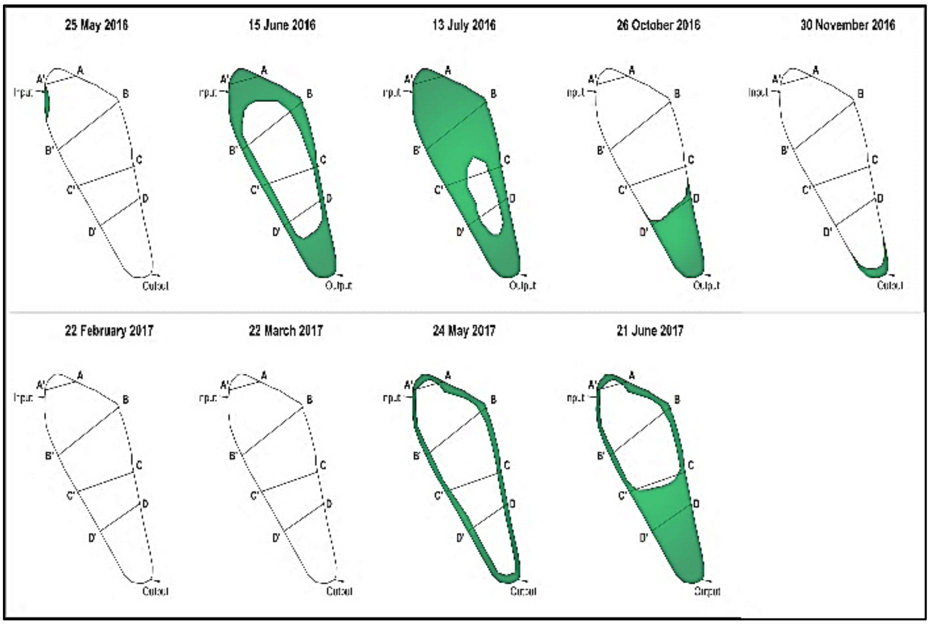

Fig. 5. Surface of Basin 1 occupied by *Lemna* during measurement campaigns

The Nitrate concentration in 22 February 2017, 22 March 2017 and 24 May 2017 measurement campaigns are higher than the others probably due to the efficiency reduction of the denitrification process, as explained before. However, in these cases the NO_3^--N reduction efficiencies were around 15% showing a finishing capacity of Basin 1 also towards to the Nitrate. The last measurement campaign (21 June 2017) shows a behavior comparable to the first period with a decrease of ammonium and nitrate in input and a good finishing capacity of Basin 1. Temperature and Dissolved Oxygen have been measured along the water column in sections B-B' and C-C' and the results shown in tables from 4 to 6.

The 25 May 2016 data (Table 4) show temperature values around twenty degrees, typical for this season, without evidence of particular trends in depth. DO confirms the aerobic condition in all the water volume and consequently nitrification process underway. Higher DO percentage in section B-B' is not due to photosynthetic activity but to the oxygen dissolved during the input. Indeed, sewage flows in Basin 1 through a free surface channel with a diameter of one meter and a length around three meters.

DO percentage on 15 June 2016 (Table 5) confirms the photosynthetic activity of phytoplankton, with higher values on the surface than on the bottom. The DO percentage does not show anoxic conditions so there is no denitrification in the bottom layer. Temperature are higher than on 25 May 2016 and approximately constant along the water column.

In addition to the presence of *Lemna* in Basin 1, the increase of Nitrogen removal efficiency observed on 13 July 2016 is also due to low DO concentration in the bottom layer of the water column (Table 6). Indeed, the anoxic conditions implied the denitrification process have taken place where the water depth was approximately more than 1.10 m. This is confirmed by the nitrate reduction efficiency around 50% (see Table 3).

The DO percentage indicates unsaturated conditions in the water column. This is due to the *Lemna* coverage, which reduced the oxygen transfer from the air to the water, and also to the reduction of the photosynthetic activity as the solar irradiation did not penetrate. Moreover, DO decrease in Summer can be also attributed to lagoon water temperature. Thus, water temperatures rise in Summer (Table 6) involves DO saturation decrease and in turn DO decrease.

Table 4. Data from the Basin 1: Temperature (t) and Dissolved Oxygen (DO) in the water column - 25 May 2016

Depth (m)	***B-B'***			***C-C'***		
	t	***DO***		***t***	***DO***	
	(°C)	***(%)***	***(mg/L)***	***(°C)***	***(%)***	***(mg/L)***
0.30	20.53	81.0	7.26	20.75	71.7	6.39
0.50	20.11	84.0	7.58	20.77	71.5	6.38
0.70	19.84	83.0	7.54	20.45	71.3	6.40
0.90	19.49	78.8	7.20	20.20	72.1	6.50
1.10	19.46	77.2	7.06	19.90	69.5	6.30
1.30	19.40	75.8	6.94	19.89	71.3	6.47
1.50	19.55	70.7	6.45	19.88	71.7	6.39

Table 5. Data from the Basin 1: Temperature (t) and Dissolved Oxygen (DO) in the water column - 15 June 2016

Depth (m)	***B-B'***			***C-C'***		
	t	***DO***		***t***	***DO***	
	(°C)	***(%)***	***(mg/L)***	***(°C)***	***(%)***	***(mg/L)***
0.30	22.12	112.4	9.77	22.30	98.0	8.53
0.50	22.10	113.9	9.90	22.30	92.1	8.02
0.70	22.10	111.0	9.65	22.23	89.0	7.74
0.90	22.10	110.8	9.63	22.22	87.2	7.56
1.10	22.11	110.1	9.57	22.21	86.2	7.47
1.30	22.14	111.1	9.65	22.08	85.2	7.38
1.50	22.12	111.7	9.70	22.01	82.7	7.16

This effect is less relevant in Spring (Tables 4-5) than in Winter. The presence of phytoplankton and organic matter, from bacterial and *Lemna* degradations, especially in Summer, enriches the water environment with organic matter which promotes bacterial growth and in turn effects DO levels.

3.2. Disinfection effect

The natural disinfection capability of Basin 1 has been tested measuring the *E. coli* concentration in different sampling points shown in Fig. 2 (i, b, c, d).

Starting from similar values in input, the overall efficiency changed significantly from 25 May 2016 to 13 July 2016 (Table 7) mainly due to the *Lemna* growth that covered the surface preventing the solar irradiation from penetrating the water. The single measurement in autumn shows a good removal efficiency (87%) but the *E. coli* concentration is not under the Italian legal thresholds yet.During Winter/Spring seasons, from 22 February 2017 to 24 May 2017, the *E. coli* removal efficiency was over 96% because there was not *Lemna* coverage and the solar irradiation permits the natural disinfection. This behaviour is evident in February, when the disinfection efficiency was 98% even if the solar radiation was not maximum. Only two output values (22 February 2017 and 24 May 2017) are under the legal thresholds showing that this goal is very hardly achievable. Finally, the last measurement campaign conducted in July (24 July 2017) shows an unexpected increase of *E. coli* concentration from input (2.4×102) to output (3.88×102).

Table 6. Data from the Basin 1: Temperature (t) and Dissolved Oxygen (DO) in the water column - 17 July 2016

Depth (m)	***B-B'***			***C-C'***		
	t	***DO***		***t***	***DO***	
	(°C)	***(%)***	***(mg/L)***	***(°C)***	***(%)***	***(mg/L)***
0.30	26.00	44.01	3.55	26.67	50.65	4.10
0.50	25.93	43.27	3.50	26.18	48.59	3.93
0.70	25.83	43.94	3.56	26.20	46.35	3.76
0.90	25.67	44.84	3.64	25.94	45.20	3.67
1.10	25.36	18.82	1.54	25.79	42.58	3.43
1.30	25.19	15.96	1.20	25.75	42.19	3.39
1.50	25.15	12.30	0.48	25.74	27.88	2.22

Table 7. *E. coli* (CFU/100 mL) in Basin 1 - standard deviations in bracket

Measurement campaign	***Sections***					***Removal efficiency***
	A-A'	***B-B'***	***C-C'***	***D-D'***	***A-A'***	
25 May 2016	2.7×10^3 (3.2×10^2)			6.77×10^2 (3.01×10^2)	3.67×10^2 (1.86×10^2)	86%
15 June 2016	1.4×10^3 (2.2×10^2)		9.27×10^2 (1.95×10^2)		5.63×10^2 (1.70×10^2)	59%
13 July 2016	2.8×10^3 (1.9×10^2)		1.85×10^3 (1.31×10^2)		1.76×10^3 (1.04×10^2)	36%
30 November 2016	3.2×10^3 (2.9×10^2)		8.77×10^2 (3.86×10^2)		4.05×10^2 (7.07×10^0)	87%
22 February 2017	2.2×10^3 (4.9×10^2)	4.33×10^2 (1.06×10^2)	3.30×10^2 (5.93×10^1)	4.75×10^1 (2.50×10^1)	3.75×10^1 (1.71×10^1)	98%
22 March 2017	1.2×10^4 (8.0×10^2)	1.57×10^3 (9.45×10^1)	8.30×10^2 (7.07×10^1)	5.23×10^2 (4.04×10^1)	2.28×10^2 (2.50×10^1)	98%
24 May 2017	7.5×10^2 (3.5×10^2)			3.00×10^1 (1.41×10^1)	3.33×10^1 (1.15×10^1)	96%
24 July 2017	2.4×10^2 (5.1×10^1)			3.88×10^2 (1.94×10^2)		-

This behavior could be due to abnormal increases of *E. coli* concentration in plant input during the previous days that should have caused *E. coli* accumulation. Consequently, Basin 1 HRT was not enough to reduce the concentration under the legal thresholds for reuse using only one lagoon.

Fig. 6 shows the results of *E. coli* degradation model implementation. The Input data (see Table 7) of each measurement campaign have been taken as starting values for the model and are shown in each picture with their respective standard deviations. Moreover, the pictures show the D-D' values with their standard deviations to compare them with the modelled values in the same section.

The model describes adequately the natural disinfection in Basin 1 even if the discrepancy from the measured values is high when all the Basin surface is covered by *Lemna*. As said before, in such cases, the solar radiation penetration is influenced by the *Lemna* thickness that in turn depends on its accumulation rate. The model did not allow to consider the *Lemna* thickness

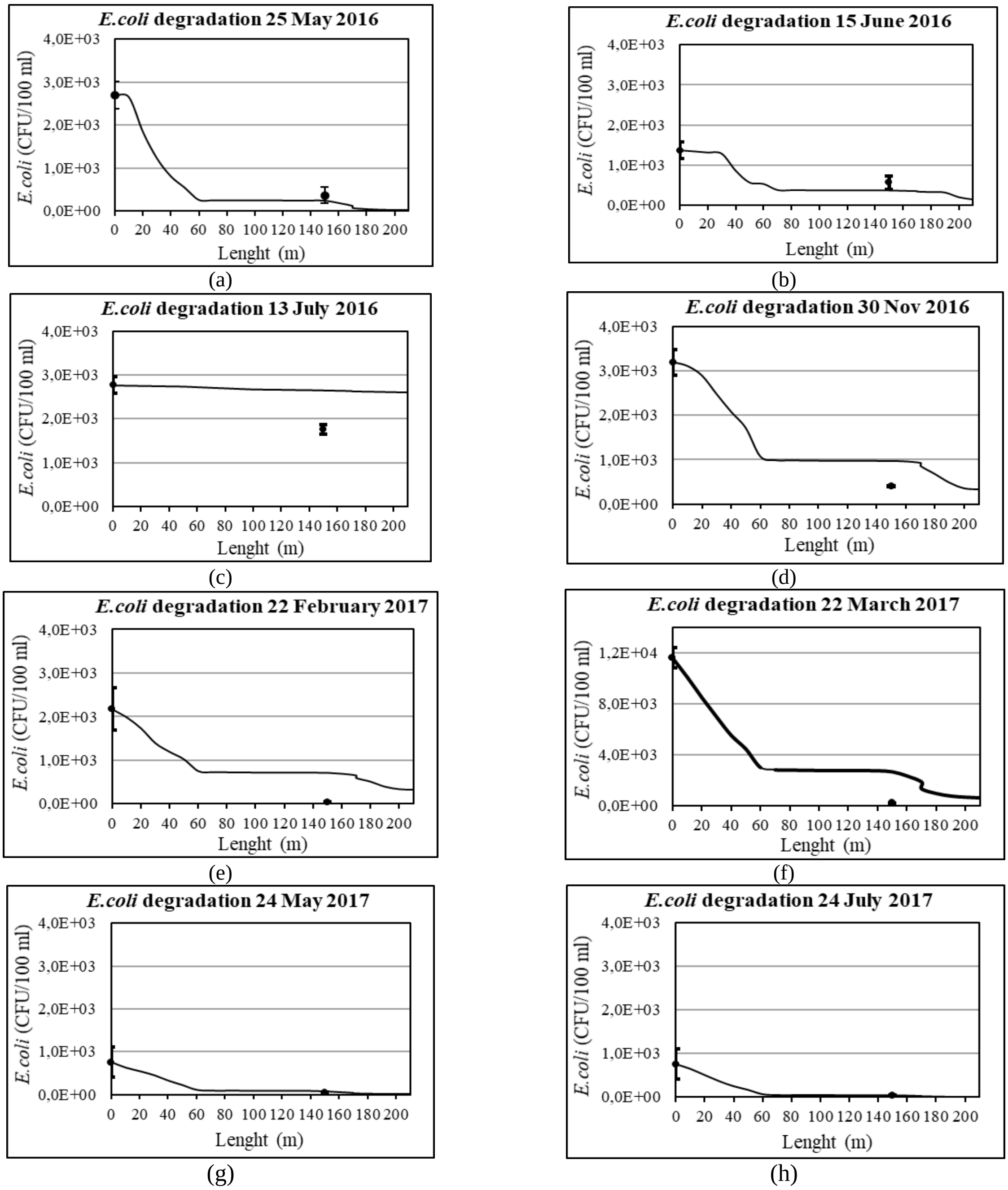

Fig. 6. Basin 1: comparison between *E. coli* measured in sections A-A' and D-D' (black points with standard deviations lines) and *E. coli* degradation modelled (black lines) in each measurement campaign: 25 May 2016 (a), 15 June 2016 (b), 13 July 2016 (c), 30 November 2016 (d), 22 February 2017 (e), 22 March 2017 (f), 24 May 2017 (g), 24 July 2017 (h)

Between 13 July 2016 and 24 July 2017, we collected samples and analysed them to obtain the *E. coli* concentration in input (PP_input) and output (PP_output) sections of the pilot plant. The samples in Basin 1 and in the pilot, plant have been collected at the same time. Table 8 shows the results in terms of *E. coli* concentration with the standard deviations in bracket and the overall *E. coli* removal efficiency. Comparing the data in Table 7 and Table 8 we note that starting from comparable input data, the natural disinfection efficiency is more than 83% in all cases except on 30 November 2016, when the input concentration is lower (1.3×10^2). Moreover, the efficiencies reached in pilot plant (Table 8) and Basin 1 (Table 7) are comparable and the output *E. coli* concentration is over the legal thresholds in all cases. However, these concentrations are not so far from the Italian thresholds and we must consider that the pilot plant surface was covered by *Lemna* in all cases, this obviously contribute to decrease the disinfection efficiency.

4. Conclusions

This study deals with the need to adequate existing WWTPs for irrigation reuse respecting the legal threshold and the water volume needs. The results from fifteen measurement campaigns on the first natural finishing basin of Santerno plant (Basin 1) have been analysed in comparison with the legal thresholds from the Italian regulation for wastewater reuse (DM 185/2003).

In the first part of the study we discussed the nitrogen compounds reduction in terms of TN and Ammonium nitrogen. Results confirmed that the irrigation purposes are achievable in terms of TN in all cases apart from two cases when the Nitrate nitrogen concentration in input was very high (20.1 mgN/L and 12.9 mgN/L). Ammonium Nitrogen was above the legal limits in all cases but a very interesting removal efficiency, up to 57%, has been registered during Spring and Summer seasons. In these cases, the Ammonium Nitrogen reduction is mainly due to high DO levels on the water column that involve high nitrification effect along it. This DO increase is due to the already oxygenated input flow and the photosynthetic extraction due to microalgae as the solar radiation is maximum that produce oxygen released in the basin. In such a case, Basin 1 behaviour can be considered as Free Water Surface lagoon. The presence of *Lemna* minor on the surface during summer reduces the solar radiation capacity to penetrate in the water column and consequently the microalgae activity, nevertheless the *Lemna* synthesis requires ammonium that is reduced on the surface layer. In such a case, Basin 1 behaviour can be considered as phyto-treatment lagoon. Furthermore, in this last case we note that denitrification conditions are possible in the deep layers of the basin (around 1.50 m), as observed during the 17 July 2016 campaign.

The second part of the study focused on the disinfection capacity of Basin 1 analysing the *Escherichia coli* concentration. Results show that *Escherichia coli* concentration in output does not permit the irrigation reuse in six cases on eight when those concentrations are under the stringent Italian legal limit (50 CFU/100mL). Anyway, we observe very interesting disinfection efficiency, up to 98% in two cases. We observed that the natural disinfection process is strongly influenced by the presence of *Lemna* on the surface as shown by the different removal efficiency along the section analysed. Moreover, the tests carried on the pilot plant shown that the top layer of the basin (around 60 cm) is the most important in terms of natural disinfection.

DO increase and E. coli decrease in Summer can also due to the presence of phytoplankton and organic matter, from bacterial and *Lemna minor* degradations which influences the bacterial activities and the disinfection capability of the system.

Finally, results show that is possible to achieve the irrigation reuse goals using existing WWTPs equipped with natural finishing lagoons only adopting adequate policies in order to manage the equilibrium between FWS lagoon and phyto-treatment due to *Lemna*. The *Lemna* extraction management in relationship with the seasonal solar radiation variations plays a key role as its presence influences both the nitrification/denitrification and disinfection processes. Those management decisions can be effectively supported by the *E. coli* degradation model tested on the full scale Basin 1 as long as the *Lemna* thickness, expressed as τ in the model, is carefully considered.

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Table 8. *E. coli* (CFU/100 mL) in pilot plant - standard deviations in bracket

Measurement campaign	***Sections***		
	PP_Input	***PP_Output***	***Removal efficiency***
13 July 2016	1.4×10^3 (4.9×10^2)	2.0×10^2 (1.5×10^2)	86%
30 November 2016	1.3×10^2 (1.7×10^2)	7.0×10^1 (5.2×10^1)	47%
22 February 2017	2.1×10^3 (2.9×10^2)	2.8×10^2 (1.1×10^2)	87%
22 March 2017	8.0×10^3 (1.1×10^3)	1.3×10^3 (2.0×10^2)	84%
24 May 2017	5.0×10^3 (1.3×10^3)	8.3×10^2 (3.1×10^2)	83%
24 July 2017	1.2×10^3 (5.5×10^2)	1.7×10^2 (7.2×10^1)	86%

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