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Integration of an innovative biological treatment with physical or chemical disinfection for wastewater reuse

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HIGHLIGHTS

- SBBGR system showed high effectiveness in removing TSS, COD and nitrogen.
- Pathogen removal by a compact system may encourage wastewater reuse in agriculture.
- SBBGR system showed disinfection efficiency higher than conventional WWTP.
- *E. coli* content after biological treatment was only 10^3 MPN/100 mL.
- *E. coli* concentration after UV or PAA disinfection was less than 10 MPN/100 mL.

GRAPHICAL ABSTRACT

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ABSTRACT

In the present paper, the effectiveness of a Sequencing Batch Biofilter Granular Reactor (SBBGR) and its integration with different disinfection strategies (UV irradiation, peracetic acid) for producing an effluent suitable for agricultural use was evaluated. The plant treated raw domestic sewage, and its performances were evaluated in terms of the removal efficiency of a wide group of physical, chemical and microbiological parameters. The SBBGR resulted really efficient in removing suspended solids, COD and nitrogen with an average effluent concentration of 5, 32 and 10 mg/L, respectively. Lower removal efficiency was observed for phosphorus with an average concentration in the effluent of 3 mg/L. Plant effluent was also characterized by an average electrical conductivity and sodium adsorption ratio of 680 μS/cm and 2.9, respectively. Therefore, according to these gross parameters, the SBBGR effluent was conformed to the national standards required in Italy for agricultural reuse. Moreover, disinfection performances of the SBBGR was higher than that of conventional municipal wastewater treatment plants and met the quality criteria suggested by WHO (*Escherichia coli* < 1000 CFU/100 mL) for agricultural reuse. In particular, the biological treatment by SBBGR removed 3.8 ± 0.4 log units of *Giardia lamblia*, 2.8 ± 0.8 log units of *E. coli*, 2.5 ± 0.7 log units of total coliforms, 2.0 ± 0.3 log units of *Clostridium perfringens*, 2.0 ± 0.4 log units of *Cryptosporidium parvum* and 1.7 ± 0.7 log units of Somatic coliphages. The investigated disinfection processes (UV and peracetic acid) resulted very effective for total coliforms, *E. coli* and somatic coliphages. In particular, a UV radiation and peracetic acid doses of 40 mJ/cm^2 and 1 mg/L respectively reduced *E. coli* content in the effluent below the limit for agricultural reuse in Italy (10 CFU/100 mL). Conversely, they were both ineffective on *C. perfringens* spores.

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1. Introduction

In past centuries, water was considered as a renewable, unlimited resource. During the last decades, however, the awareness that high quality water is not unlimited has started to arise in both people and government organizations around the world. Moreover, the increase of the word population and occurring climate changes suggest the need for a more rational use of water resources.

A recent study by the European Environment Agency (EEA—European Environment Agency, 2012) evaluated the annual total water withdrawal of European countries with respect to total freshwater resources. The results indicated that, for the year 2010, about 50% of European countries were characterized by a water stress index higher than 10%, indicating that water availability is becoming a constraint for the countries' development. These studies are based on average data coming from each country, and they do not take account of regional differences within each country. Therefore, the local situation could be generally worse than that reported (i.e., based on average values).

Agriculture plays an important role in water consumption, representing the major freshwater use in most countries. It accounts for about 70% of global freshwater withdrawals (Alexandratos and Bruinsma, 2012; Levine and Asano, 2004). According to this, the reuse of treated wastewater in agriculture could provide an effective alternative for meeting agriculture's demand and also increase freshwater resources for other needs. To comply with agricultural water demand, a reduction of the centralization level of wastewater systems would be required. In fact, decentralized plants are more flexible and they might lead to the treatment and reuse of water in the same area where it is consumed.

Among the new systems recently proposed that can comply with this request, the Sequencing Batch Biofilter Granular Reactor (SBBGR), developed by the Water Research Institute (IRSA) of the Italian National Research Council (CNR) during the last decade, seems to be somewhat interesting. SBBGR belongs to attached biomass systems operating in a fill-and-draw mode. Therefore, SBBGR combines the advantages of attached biomass systems (i.e., greater robustness and compactness) with those of periodic systems (i.e., greater flexibility and stability).

Therefore SBBGR system increases the simplification of the treatment scheme for treating and reusing municipal wastewater and improves the management of water demand and supply. Furthermore, it is able to reduce the quantity of sludge usually produced during wastewater treatment, and this represents one of the most concerning issues in non-centralized plants because of the absence of sludge-treatment facilities. SBBGR treatment can also be chemically or physically enhanced when a high effluent quality is needed for agriculture reuse.

The effectiveness of the SBBGR system has already been investigated on different kinds of wastewater (municipal and industrial effluents) with the aim of producing an effluent suitable for direct discharge to the environment. In these applications, the SBBGR system has always shown high and stable removal efficiencies, producing a very low quantity of solid residues (Di Iaconi et al., 2004; Di Iaconi et al., 2010; Di Iaconi et al., 2011; Di Iaconi et al., 2014; Lotito et al., 2012). In the present study, the effectiveness of the SBBGR system for producing an effluent to be reused in agriculture is evaluated. As is known, wastewater reuse in agricultural irrigation poses serious risks to the environment and public health, and, for this reason, specific levels of effluent quality are required. It must be pointed out, however, that, despite the high interest in wastewater reuse, common quality standards for wastewater reuse in agriculture are still missing at the European level. In fact, while several countries have limited the quality criteria to a few main chemical and physical parameters (i.e., COD, suspended solids, total coliforms, faecal coliforms or *Escherichia coli*), there are countries that have adopted more stringent parameters, including emerging pollutants, metals and different kinds of microbial indicators (Brissaud, 2006; Li et al., 2009; Salgot et al., 2006).

In the present study, a larger group of physical, chemical and microbiological parameters was considered for evaluating the effectiveness of the SBBGR system for municipal wastewater treatment and reuse. SBBGR enhancement with chemical (by peracetic acid, PAA) or physical (by UV radiation) disinfection was also evaluated.

The monitored physical and chemical parameters included all those indicated by the Italian regulation (details on Italian limits for wastewater reuse are available as Supplementary material — Table S.1). Differently, the analysis of microbiological parameters was not limited to the two parameters required by this regulation (*E. coli* and *Salmonella*). Indeed, the microbial indicators were chosen considering that most human pathogens that could be derived from the reuse of wastewater belong to the domains of bacteria, viruses and protozoa, and these microorganisms are characterized by different physiological characteristics and consequently different survival rates in wastewater treatment. Therefore, microorganisms belonging to these domains were selected to evaluate the quality of the treated wastewater. The selected microbial indicators were total coliforms, *E. coli* and *Salmonella* (representative of bacteria), *Clostridium perfringens* spores (representative of spore-forming bacteria), Somatic coliphages (representative of viruses) and *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts (representative of protozoa).

2. Materials and methods

2.1. Biological treatment

Biological treatment was performed by a laboratory scale SBBGR reactor as shown in Fig. 1. It was based on a cylindrical Plexiglass reactor with a working volume of 21 L filled to 40% with plastic wheel-shaped elements packed between two sieves. The reactor was equipped with a filling pump for feeding the reactor from a wastewater storage tank, an aerator for air supply in the liquid phase over the bed, a recycle pump for allowing (by means of an external loop) the liquid and dissolved oxygen to flow through the reactor bed and a motorized valve for effluent discharge.

The operation of the biological system was automated by a programmable logic controller (PLC) and based on a succession of 8 h treatment

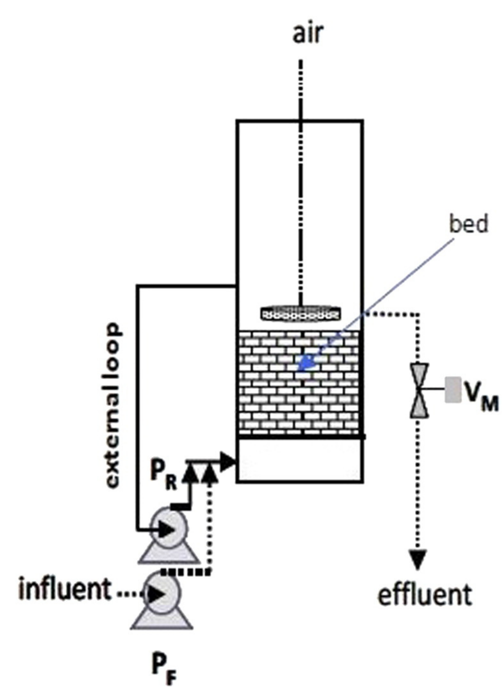

Fig. 1. Sketch of the lab-scale SBBGR system used for biological treatment. P_R: recirculation pump; P_F: feeding pump; V_M: electric valve for effluent discharge.

cycles consisting of three consecutive phases. During the filling phase, 8 L of raw wastewater were added to the liquid remaining in the reactor from the previous treatment cycle. In the biological degradation phase, the liquid in the reactor was continuously aerated and recycled through the biomass supporting material. Finally, 8 L of effluent were discharged from the reactor via a port located in the liquid phase over the bed. Therefore, the SBBGR system operated with a hydraulic residence time of 21 h.

A pressure meter, set at the bottom of the reactor, measured on-line biofilter head losses due to the biomass growth and retained suspended solids present in the influent during the operation of the plant. When a fixed set value of head loss was reached, a washing step was carried out by compressed air. Washing water was collected and measured (in terms of volume and total and volatile suspended solids content) for calculating the specific sludge production and stabilization levels. After the start-up period the experimental campaign lasted about nine months.

Raw domestic sewage coming from a small community of Bari, a southern Italy city, was used for feeding the plant. The sewage was only subjected to screening in order to remove coarse materials. The composition of the sewage is reported in Table 1.

2.2. Tertiary disinfection

Tertiary disinfection tests were carried out on the SBBGR effluent by using two strategies: physical disinfection by UV radiation or chemical disinfection by the addition of peracetic acid (PAA).

The UV disinfection was conducted with a flow-through annular photoreactor (volume: 0.5 L) equipped with a low pressure mercury vapour UV lamp (MTL844-G model by Helios Italquartz srl Milano, Italy) that emits at 254 nm (input power: 40 W). UV fluence rate, determined by uridine actinometry (Jin et al., 2006), was 40 mW/cm^2. The plant effluent was collected and flowed through the UV lamp by means of a submerged pump having a flow rate of 1800 L/h in order to obtain an exposure time of 1 s. Therefore, a UV dose (obtained by multiplying the fluence rate by exposure time) of 40 mJ/cm^2 was applied.

Chemical disinfection tests were carried out by adding a known volume of a commercial PAA solution (15 g PAA/L and 10 g H_2O_2/L, OXIFIBRO, Nuova Farmec, Italy) to a fixed volume of SBBGR effluent. The solution was maintained under stirring for 30 min and then the microbial analysis was performed. Considering the high cost of PAA and the increase in COD concentration caused by its addition to the effluent, three PAA concentrations were tested (1, 2 and 3 mg/L). Taking into account the low PAA concentrations used, the residual disinfectant was not quenched by the addition of sodium thiosulphate (for PAA removal) and bovine catalase (for hydrogen peroxide removal) as reported in the literature (Dell'Erba et al., 2004; Koivunen and Heinonen-Tanski, 2005; Rossi et al., 2007).

Each experiment was carried out in duplicate and during the experimental campaign six set of disinfection experiments were performed.

Table 1
Performances of biological treatment by the SBBGR system in terms of influent and effluent concentrations and their relative removal of the main physical and chemical gross parameters (number of analysed samples for SAR: 6; number of analysed samples for the other parameters: 80).

Parameter		Mean value ± standard deviation
pH	Influent	7.5 ± 0.1
	Effluent	7.8 ± 0.2
Conductivity	Influent [μS/cm]	891 ± 88
	Effluent [μS/cm]	680 ± 40
SAR	Influent	2.7 ± 0.7
	Effluent	2.9 ± 1.3
TSS	Influent [mg/L]	215 ± 103
	Effluent [mg/L]	5 ± 4
	Removal efficiency [%]	97 ± 2
VSS	Influent [mg/L]	168 ± 89
	Effluent [mg/L]	3 ± 3
	Removal efficiency [%]	98 ± 1
COD	Influent [mg/L]	492 ± 146
	Effluent [mg/L]	32 ± 9
	Removal efficiency [%]	93 ± 2
BOD5	Influent [mg/L]	285 ± 86
	Effluent [mg/L]	1 ± 2
	Removal efficiency [%]	99 ± 1
TKN	Influent [mg/L]	57 ± 13
	Effluent [mg/L]	2 ± 1
	Removal efficiency [%]	96 ± 1
NH3	Influent [mgN/L]	42 ± 11
	Effluent [mgN/L]	0 ± 1
	Removal efficiency [%]	99 ± 4
TN	Influent [mg/L]	58 ± 13
	Effluent [mg/L]	10 ± 4
	Removal efficiency [%]	83 ± 6
P-tot	Influent [mg/L]	4 ± 1
	Effluent [mg/L]	3 ± 1
	Removal efficiency [%]	25 ± 14

SAR: sodium adsorption ration.

2.3. Process performance evaluation

The process performances were evaluated in terms of treatment efficiency (by measuring the main physical and chemical gross parameters) and disinfection efficiency (by measuring the microbial indicators of faecal contamination and index pathogens).

The main physical and chemical gross parameters included pH, conductivity, chemical oxygen demand (COD), ammonia (NH_3), nitrites (NO_2^-), nitrates (NO_3^-), total Kjeldahl nitrogen (TKN), total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and volatile suspended solids (VSS). Apart from pH and conductivity (measured by selective probes) and TKN (calculated as the difference between TN and oxidized nitrogen), the other parameters were determined according to the standard methods (APHA et al., 2005). These parameters were monitored in SBBGR influent and effluent twice a week. The removal efficiencies of the physical and chemical gross parameters were calculated as a percentage reduction in the value between the influent and effluent samples.

To better evaluate the suitability of the treated wastewater for agricultural use, the sodium adsorption ratio (SAR) in the plant influent and effluent was monitored six times during the experimental campaign (twice for each season). This parameter expresses the relative activity of sodium ions in the exchange reaction with the soil according to the following equation:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}.$$

High concentrations of ions in water affect the permeability of soil and cause infiltration problems. This is due to the fact that sodium is able to replace calcium and magnesium in the soil, leading to the dispersion of soil particles. The soil becomes hard and compact when dry and reduces infiltration rates of water and air into the soil, affecting its structure. The analysis of sodium, calcium and magnesium was carried out by means of atomic absorption spectrophotometer analysis.

The disinfection efficiency was evaluated by measuring *E. coli*, total coliforms, Somatic coliphages, *C. perfringens*, *Salmonella*, *C. parvum* oocysts and *G. lamblia* cysts in influent and biological effluent samples and after tertiary disinfection samples. The removal efficiencies were expressed in terms of log units removed (LUR) according to the following equation:

$$LUR = \log c_a / c_b$$

where c_a and c_b are the concentrations of the specific microbial indicator after and before the specific treatment.

Total coliforms and *E. coli* concentrations were determined by using IDEXX Colilert-18 and Quanti-Trays/2000 according to the manufacturer instructions (IDEXX Laboratories, Inc., 2013). This is based on the most probable number (MPN) technique, and is an enzyme-based assay. Somatic coliphages were detected according to the ISO 10705-2:2000 method. Each tested dilution was analysed at least in quadruplicate. For SBBGR effluent and disinfected samples (UV and PAA), at least 10 mL of the original sample were scrutinized, and the results expressed in terms of plaque-forming units per unit volume (PFU/100 mL). *Salmonella* was detected according to the ISO 19250:2010 method in 1 L of the sample. Spores of *C. perfringens* were quantified according to the ISO 7937:2004 method. The analysis was performed at least in duplicate, and the results expressed in terms of colony forming units per unit volume (CFU/100 mL). Pathogenic protozoan parasites, *C. parvum* and *G. lamblia*, were detected by US EPA Method 1623. The analysis was conducted on 250 ml of influent wastewater and 1 L of treated wastewater respectively. The results were expressed in terms of oocysts/L and cysts/L respectively.

In addition to the parameters reported above, a wide group of additional parameters required by Italian regulation for wastewater reuse were occasionally monitored (two times during the whole experimental period). These parameters included metals, cyanides, sulphur, chlorines, fluorides, animal and vegetable fats and oils, mineral oils, phenols, chlorinated solvents, trihalomethanes, aromatic organic solvents, organic nitrogenous solvents, surfactants and pesticides. The analysis of these compounds was conducted according to the standard methods. It must be pointed out that most European regulations concerning wastewater reuse do not set any limit for these compounds. The abovementioned additional parameters were monitored to comply the Italian regulation for agricultural and also according to the growing concern about their release and accumulation in the environment.

3. Results and discussion

3.1. Biological treatment

3.1.1. Depuration performances

The performances of the SBBGR plant recorded during nine months of operation at a hydraulic residence time of 21 h are reported in Table 1. In particular, this table reports the influent and effluent concentrations of the main physical and chemical gross parameters with their relative removal efficiency.

Taking into account the final destination of the treated effluent (i.e., its reuse in agriculture), the values of electrical conductivity and the sodium adsorption ratio are of great importance. In fact, according to the literature (Rahimi et al., 2000; Travis et al., 2010), high values of these parameters (EC > 3000 μS/cm; SAR > 10) can greatly affect plant growth (because of salinity stress) and soil properties because of a reduction of soil tensile strength (SAR). Indeed, several countries (such as Italy, Jordan, Spain, and Turkey) have set limits for EC and SAR in the case of wastewater reuse in agriculture. However, the data reported in Table 1 clearly show that the SBBGR effluent was characterized by EC and SAR values always lower than 1000 μS/cm and 4 respectively, values compatible with crop irrigation and complying with current Italian legislation requirements.

TSS and COD are two other key parameters to assess the suitability of treated wastewater for agriculture reuse. In fact, high concentrations of suspended solids can lead to severe damage of the irrigation network, especially in the case of drip irrigation systems. In fact, suspended solids can accumulate in an irrigation tubing system and water diffusers, leading to the clogging of the irrigation network. Similarly, a high presence of biodegradable COD could cause irrigation network clogging due to microbial growth within the system. Looking at Table 1, it is possible to observe, however, that the plant performed very well in removing both these parameters. TSS removal efficiency was always higher than 89% (on average 97%) with residual concentration in the effluent of 5 mg/L, thus complying even with the very stringent Italian regulation for agricultural reuse (i.e., 10 mg/L; D.M. 185/2003).

Also, for COD, the plant performances were not influenced by the wide fluctuation in the wastewater composition. This was reflected in the volumetric organic loading rate applied with values up to 2.3 $gCOD/L_{bed}$ days. The plant successfully removed COD with a removal efficiency always higher than 87% (on average 93%) and an average effluent concentration of 32 mg/L. These performances allowed the Italian limit of COD (i.e., 100 mg/L) to be continuously met (the COD concentration in the effluent never exceeded the value of 62 mg/L).

The high performances and stability of SBBGR in removing suspended solids and COD can be ascribed to the particular feature of the system, a feature consisting of biomass confined in a fixed filling material which acts as a filtering media for removing suspended particulate matter. The latter is initially physically entrapped in the reactor bed and then successively decomposed in small soluble compounds by the lytic enzymes produced by the biomass and utilized for microbial growth.

Regarding nitrogen, the plant was able to remove about 83% of the influent nitrogen content with residual concentrations always lower than the Italian limit for wastewater reuse (i.e., 15 mg/L). This was due to the presence of a nitrification and denitrification process that was somewhat stable. In fact, TKN and ammonia removal efficiencies as high as 96% and 99% respectively were constantly obtained as confirmed by the low deviation standard values reported in Table 1. Furthermore, the concentration profiles of nitrogen species recorded during a typical treatment cycle (data not shown) have indicated that it is a simultaneous nitrification and denitrification process. This ability of the system is ascribed to the high value of biomass concentration present in the reactor bed, which allows the cohabitation of nitrifying and denitrifying bacteria in the biomass layers as reported in De Sanctis et al. (2010). Phosphorous was only slightly removed from the plant with an average effluent concentration of 3 mg/L. This value exceeds the Italian limit for wastewater reuse set at 2 mg/L. The regulation allows, however, increasing this value up to 10 mg/L in the case of agricultural reuse on the basis of regional regulations. Phosphorous (like nitrogen) is a fundamental nutrient for crops, and it is generally supplied to plants by adding external sources; therefore, the presence of some residual phosphorous in the effluent should be considered as an added value. Moreover, different from TSS and COD, the presence of phosphorous in the irrigation water is not associated with any drawback. The observed low phosphorus removal level is in accordance with the low sludge production value recorded for the SBBGR system (i.e., up to four times lower than that reported for conventional wastewater treatment plants based on activated sludge systems). In addition to the low sludge production, the sludge result even stabilized, thus requiring no further biological treatment. These findings can be ascribed to the high age of the biomass that can be achieved by the SBBGR system thanks to its particular features.

As reported in Section 2.3, a wide group of additional parameters (such as metals, cyanides, sulphur, chlorines, fluorides, animal and vegetable fats and oils, mineral oils, phenols, chlorinated solvents, trihalomethanes, aromatic organic solvents, organic nitrogenous solvents, surfactants and pesticides) required by Italian regulation for wastewater reuse were occasionally monitored. Most of these compounds were not detected (see Supplementary material — Table S.2), or they were present in traces in accordance with the wastewater type (i.e., domestic sewage). However, the level of these parameters in the effluent was always much lower than the Italian limits for wastewater reuse. The monitored compounds included several metals and xenobiotic substances generally classified as emerging pollutants. Actually, most regulations about water reuse do not establish a limit for these compounds; nevertheless, they were monitored according to the growing concern about their release and accumulation in the environment.

3.1.2. Disinfection performances.

The ability of the SBBGR system in removing faecal indicators and index pathogens was evaluated by monitoring total coliforms, *E. coli*, Somatic coliphages, *C. perfringens*, *Salmonella*, *C. parvum* and *G. lamblia*.

The disinfection performances of the biological treatment by SBBGR are reported in Table 2. In particular, the table reports the concentrations of the pathogenic indicators in the plant influent and effluent and their relative LUR values. Looking at the data reported in this table, it is possible to observe that the biological treatment result was effective in removing all the monitored microorganisms, especially with regard to bacteria and protozoa, showing removals higher than two logarithmic units. In detail, the plant cut down about 2.5 log units of total coliforms. The disinfection efficiency results of the plant are more valuable if compared with the performances obtained in large wastewater treatment plants based on conventional technologies and reported in the literature. In fact, Koivunen et al. (2003) report a total coliforms removal between 2 and 3 log units in four Finnish treatment plants. However, it should be highlighted that this value refers to the whole wastewater line (i.e., preliminary, primary and secondary stages) of the treatment plant. Similarly, Wen et al. (2009) observed about a 2.3 log unit removal of total coliforms in a full scale Bolivar wastewater treatment plant (WWTP) based on an activated sludge system in a nutrient removal configuration. The same authors evaluated also the disinfection efficiency of three laboratory scale SBRs. The laboratory scale plants were operated in order to simulate the full scale plant and they were fed with primary settled wastewater from the Bolivar WWTP. Treatment cycle included an aerobic and anaerobic phase and the sludge age was maintained at 20 days. The results indicated that the laboratory scale plants were characterized by almost the same disinfection efficiency of the full scale plant ensuring a removal of about 2.4 log units of total coliforms.

Lower removal efficiency (i.e., a removal of 1–2 log units of total coliforms and *E. coli*.) was observed by de Nardi et al. (2011) by using a lab-scale SBR fed with the effluent of an UASB (anaerobic sludge blanket) treating poultry slaughterhouse wastewater.

Therefore, these results clearly show that SBBGR is able to offer, in a single step, almost the same removal of total coliforms obtained by conventional plants through several steps.

Table 2
Disinfection efficiency of the SBBGR system in terms of influent and effluent concentrations and relative LUR of the selected pathogenic indicators (number of analysed samples: 12).

Parameter		Mean value[a] ± standard deviation
Total coliforms	Influent [MPN/100 mL]	$1.1 \pm 1.5 \cdot 10^7$
	Effluent [MPN/100 mL]	$2.1 \pm 1.5 \cdot 10^4$
	LUR	2.5 ± 0.7
Escherichia coli	Influent [MPN/100 mL]	$2.1 \pm 3.9 \cdot 10^6$
	Effluent [MPN/100 mL]	$2.0 \pm 3.0 \cdot 10^3$
	LUR	2.8 ± 0.8
Clostridium perfringens	Influent [CFU/100 mL]	$2.4 \pm 2.3 \cdot 10^5$
	Effluent [CFU/100 mL]	$2.4 \pm 2.0 \cdot 10^3$
	LUR	2.0 ± 0.3
Somatic coliphages	Influent [PFU/100 mL]	$1.4 \pm 1.8 \cdot 10^4$
	Effluent [PFU/100 mL]	$4.8 \pm 5.6 \cdot 10^2$
	LUR	1.7 ± 0.7
Cryptosporidium parvum oocysts	Influent [oocysts/L]	$2.3 \pm 3.1 \cdot 10^2$
	Effluent [oocysts/L]	1.3 ± 0.7
	LUR	2.0 ± 0.4
Giardia lamblia cysts	Influent [cysts/L]	$4.1 \pm 6.2 \cdot 10^4$
	Effluent [cysts/L]	5.2 ± 6.2
	LUR	3.8 ± 0.4
Salmonella	Influent	Present
	Effluent	Not present

LUR: log units removed; MPN: most probable number; CFU: colony forming unit; PFU: plaque forming unit.
[a] For negative samples the detection limits were used.

SBBGR performed even better against *E. coli*, allowing removal on average of 2.8 log units and leading to an average concentration in the effluent of $2 \cdot 10^3$ MPN/100 mL and a concentration even lower than 10^3 MPN/100 mL in 70% of the samples. The *E. coli* concentration in the plant effluent was about 10–100 times lower than that usually reported in conventional WWTPs. In fact, Rossi et al. (2007) and De Luca et al. (2013) have reported an average *E. coli* concentration in the effluent of three Italian WWTPs based on activated sludge technology of about $5 \cdot 10^4$ CFU/100 mL. Carducci et al. (2008) reported even higher levels of *E. coli* (of about 10^5 MPN/100 mL) in the effluent of another Italian WWTP.

Similarly, the previously cited study by Wen et al. (2009) reported *E. coli* concentrations ranging between $7.9 \cdot 10^4$ and $3.0 \cdot 10^5$ in the effluent of three laboratory scale SBRs. In particular the plants removed about 2.1 log units of *E. coli* (i.e., a value lower than that obtained for SBBGR).

Therefore, SBBGR appears to be much more efficient than conventional WWTP in *E. coli* removal. Moreover, the SBBGR effluent met the Italian limit for discharge in water bodies ($5 \cdot 10^3$ CFU/100 mL). This means that, if, in some period, the water reclaimed is not required by agriculture or other users, the treated wastewater could also be directly discharged into water bodies without any further disinfection step. Effluents from conventional WWTP would require a tertiary disinfection process to allow their discharge. SBBGR effluent quality is also almost in line with that indicated by the WHO guidelines for agricultural reuse (lower than 10^3 CFU/100 mL; WHO, 2006), though it still does not meet the extremely low Italian limit for wastewater reuse (i.e., 10 CFU/100 mL).

SBBGR efficiently removed *C. perfringens* also with an average removal of 2.0 log units. This microorganism is representative of spore-forming bacteria, which are generally considered more resistant than other bacteria to biological, physical and chemical disinfection treatments. In fact, in a study conducted on six municipal full scale WWTP located in Argentina, France and Spain, Lucena et al. (2004) found that primary and secondary units of the plants were able to remove only 1 log unit of this microorganism. Similar findings were reported by Wen et al. (2009) for a similar WWTP in Bolivar. Differently a higher removal (about 2 log units) was observed by Wery et al. (2008) in a more complex municipal wastewater treatment plant comprised of sections for pre-treatment (screening, de-oiling and sand removal), an aerated section for fat treatment, biological basins for denitrification, nitrification and phosphorus removal and a section for secondary settling. Therefore, as already observed for *E. coli*, despite its compactness and the absence of any pre-treatment that could reduce the concentration of *C. perfringens* coming in to the plant, SBBGR showed removal efficiencies of this bacteria higher (or at least comparable to) than those of conventional WWTPs usually obtained by using several treatment steps. The performance of SBBGR in removing *C. perfringens* should be considered of great relevance since these microorganisms are somewhat resistant to physical and chemical disinfection. In fact, the high resistance of *C. perfringens* to disinfection was evaluated in a study by Gehr et al. (2003) on municipal wastewater after enhanced primary treatment. *C. perfringens* were almost completely resistant to a peracetic acid dosage between 1.5 and 6 mg/L, whereas 30–40 mJ/cm^2 of a UV dose and an ozone dose of about 40 mg/L were required to obtain a 1 log unit of removal. Therefore, the reduction of *C. perfringens* from secondary plant effluents by these strategies would be quite expensive. At present, a limit for this microorganism in the case of agricultural reuse, which fixed the values of 100 or 1000 CFU/100 mL in the case of unrestricted or restricted irrigation respectively, has been set only by Spain (Salgot et al., 2006). Indeed, the SBBGR effluent quality was characterized by an average *C. perfringens* concentration of $2.4 \pm 2.0 \cdot 10^3$ CFU/100 mL, a value that is very close to Spain's requirement for the direct reuse of treated wastewater.

The SBBGR was also able to ensure a 1.7 log unit of removal of somatic coliphages with an average effluent concentration of 4.8 ±

$5.6 \cdot 10^2$ PFU/100 mL. This removal is comparable with that one obtained in conventional WWTPs. In fact, Lucena et al. (2004) found that a municipal full scale WWTP based on preliminary, primary and secondary treatment units can remove about 1.5 log units of these microorganisms. Notably, according to Gantzer et al. (1998) enteroviruses are not detected when the concentration of somatic coliphages is lower than 10^3 PFU/100 mL. This finding suggests that also these pathogenic viruses might be reduced to non-detectable level by the SBBGR process.

The SBBGR treatment was extremely effective against *C. parvum* and *G. lamblia*. These pathogenic protozoa were always detected in the wastewater fed to SBBGR with an average concentration of $2.3 \pm 3.1 \cdot 10^2$ and $4.1 \pm 6.2 \cdot 10^4$ oocysts and cysts/L respectively. Indeed, the presence of these protozoa was detected only in 40% and 67% of treated wastewater samples respectively. Moreover, *C. parvum* and *G. lamblia* concentrations were always lower than 3 and 12 oocysts and cysts/L, respectively. Therefore, SBBGR treatment was more efficient than conventional processes in removing these pathogens. Indeed, the ability of these microorganisms to generate cysts makes them more resistant to adverse environmental conditions. Castro-Hermida et al. (2008) carried out a study on twelve Spanish municipal WWTPs to investigate the removal of these two protozoa in WWTPs. Eight plants included primary and secondary treatments (based on activated sludge systems), three also included a tertiary treatment by UV irradiation and one was based on biological aerated filter technology. The authors observed that all plants were ineffective against both protozoa, leading to a removal of less than 1 log unit of *Cryptosporidium* and *Giardia*. Better results on *G. lamblia* removal were observed by Cacciò et al. (2003) who evaluated the performances of four Italian municipal WWTPs. The authors observed an average removal efficiency ranging from 87 to 98% depending on the plant configuration. In particular, better results were obtained for secondary treatment units aerated by pure oxygen (94.5%) or in presence of filtration (60 μm pore) combined with chemical disinfection by 4 mg/L of peracetic acid (98.4%). Therefore, the SBBGR system seems to offer better performances in removing *Cryptosporidium* and *Giardia* compared to conventional WWTPs. This acquires greater relevance if one considers that these protozoa are particularly resistant to chemical and physical disinfection as observed in the previously cited research (Cacciò et al., 2003; Castro-Hermida et al., 2008).

As already observed for *C. perfringens*, the presence of *Cryptosporidium* and *Giardia* in treated wastewater for agricultural reuse is regulated only in Spain, where a limit of 200 cysts/L in the case of unrestricted agricultural reuse was established (Salgot et al., 2006). Therefore, the level of *Cryptosporidium* and *Giardia* detected in the SBBGR effluent (1.3 ± 0.7 oocysts/L and 5.2 ± 6.2 cysts/L respectively) would allow the direct reuse of the treated wastewater in agriculture in Spain.

Finally also, the pathogenic bacterium *Salmonella* was efficiently removed during the treatment by SBBGR. This bacteria was detected in about 20% of the analysed influent samples but it was never detected in the effluent of the plant.

In conclusion, the results obtained clearly show that SBBGR acts also as a good disinfection system. Despite its simple scheme and compactness, the effluent quality of the SBBGR system is higher than that of conventional activated sludge systems. Indeed, the biological treatment by SBBGR allows reaching an effluent quality compatible or very near to the standards required by several countries for the agricultural reuse of treated wastewater. Nevertheless, the SBBGR effluent still does not meet the very strict limit established by Italian regulation for *E. coli* (10 CFU/100 mL). Therefore, a further disinfection treatment would be required for wastewater reuse in Italy. A lower disinfectant dose is, however, expected in this case.

3.2. Tertiary disinfection.

As reported in Section 2.2, the biological disinfection was enhanced by combining the SBBGR treatment with a traditional chemical (by peracetic acid) and physical (by UV radiation) disinfection process in order to further reduce the presence of the investigated faecal contamination and pathogenic indicators and to meet the very stringent Italian limits for wastewater reuse.

On the basis of the high quality of the SBBGR effluent, a UV radiation dose of 40 mJ/cm^2 and three dosages of PAA (i.e., 1, 2 and 3 mg/L) were tested. Table 3 reports the concentration of the investigated microbiological parameters after the physical and chemical disinfection treatment and their removal in terms of logarithmic units. Considering the almost complete removal of the protozoa (*C. parvum* and *G. lamblia*) and the complete removal of *Salmonella* obtained during the biological treatment, their presence was not further investigated after the physical and chemical disinfection processes.

The results obtained for total coliforms and *E. coli* indicate that both investigated disinfection processes (i.e., UV and PAA) were very efficient in removing these groups of bacteria. In particular, the UV treatment was able to remove about 3 log units of total coliforms and reduce the number of *E. coli* to less than 4 MPN/100 mL (on average), thus meeting the Italian limit for unrestricted agricultural wastewater reuse (i.e., ≤10 CFU/100 mL). As far as peracetic acid disinfection is concerned, the results show good removal efficiencies for all the investigated doses. Furthermore, as expected, the disinfection efficiency increases as the PAA dose becomes larger. Interestingly, compared to the physical disinfection, the chemical disinfection by PAA shows almost the same efficiency on total coliforms while it was more effective against *E. coli*. Indeed, 1 mg/L of PAA was able to almost completely remove *E. coli* with a residual concentration of 1.4 MPN/100 mL (*E. coli* was detected only in 20% of the samples), thus complying the Italian limit for unrestricted agricultural wastewater reuse.

It should be noted that the UV and PAA doses used in this study are lower than those usually reported in the literature. In fact, Gori and Caretti (2008), in a study on two traditional municipal WWTPs (composed of preliminary, primary, secondary and sand filtration treatments) aimed at investigating the effectiveness of PAA disinfection followed by UV radiation, found that 2–4 mg/L of PAA and a UV dose of 165–170 mJ/cm^2 were required for meeting the Italian limit of 10 CFU/100 mL of *E. coli*. These results confirm the higher quality of the SBBGR effluent compared to that of conventional WWTPs.

The data in Table 3 show that somatic coliphages were also quite sensitive to both physical and chemical disinfection but were less sensitive than *E. coli*. In particular, the UV radiation was more effective, ensuring an average removal higher than 1.6 log units of coliphages. Indeed, *coliphages* were detected in only 20% of the analysed samples. Differently, chemical disinfection removed on average from 0.9 to 1.2 log units on the basis of the applied PAA dose. The higher variability of the results obtained by using PAA suggests that at least 3 mg/L of PAA should be used to ensure a somatic coliphage removal comparable with that obtained by UV irradiation. It is interesting to observe, however, that 1 mg/L of PAA is able to reduce the coliphages concentration in the effluent to below 80 PFU/100 mL, thus complying with the Spanish limit set for this microorganism for unrestricted agricultural reuse (<100 PFU/100 mL) that is at present the only one officially established.

The higher sensitivity of coliphages to UV irradiation than to PAA is in agreement with the results reported by Gehr et al. (2003), which compared the disinfection efficiency of UV irradiation, PAA and ozone. The authors found that the removal of 1 log unit of MS2-coliphages could be achieved with a UV dose of 20 mJ/cm^2, and the removal of this virus linearly increased with the increase of the UV dose. Differently, the same virus removal of about 1 log unit was obtained by using PAA doses in the range 1.5–4.5 mg/L.

Unlike the other investigated parameters, *C. perfringens* were resistant to UV and PAA disinfection. However, it should be considered that *C. perfringens*, being spore forming microorganisms, persist in the environment longer and are more resistant to disinfection processes, compared to the other investigated pathogenic indicators. Indeed, according to the literature (Gehr et al., 2003), *C. perfringens* is characterized by a quite high resistance to both UV radiation and peracetic acid.

Table 3
Residual concentrations and relative LUR of the investigated microbial parameters recorded during the disinfection tests on the SBBGR effluent (number of analysed samples: 12).

Parameter		Mean value[a] ± standard deviation			
		UV	Peracetic acid		
		40 mJ/cm^2	1 mg/L	2 mg/L	3 mg/L
Total coliforms	Effluent [MPN/100 mL]	0.7 ± 1.0·10^2	2.0 ± 4.0·10^2	6.5 ± 11.0	1.2 ± 0.5
	LUR	2.8 ± 0.9	2.8 ± 1.0	3.8 ± 0.6	4.2 ± 0.4
Escherichia coli	Effluent [MPN/100 mL]	3.4 ± 4.8	1.4 ± 0.9	1.0 ± 0.0	1.0 ± 0.0
	LUR	2.6 ± 1.0	2.9 ± 0.6	2.9 ± 0.7	3.0 ± 0.7
Somatic coliphages	Effluent [PFU/100 mL]	1.8 ± 1.8·10	4.7 ± 3.5·10	2.7 ± 2.1·10	2.3 ± 2.3·10
	LUR	1.6 ± 1.0	0.9 ± 0.3	1.1 ± 0.6	1.2 ± 0.8
Clostridium perfringens	Effluent [CFU/100 mL]	1.6 ± 1.6·10^3	2.1 ± 1.7·10^3	1.4 ± 1.5·10^3	1.4 ± 1.0·10^3
	LUR	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.2

LUR: log units removed; MPN: most probable number; CFU: colony forming unit; PFU: plaque forming unit.
[a] For negative samples the detection limits were used.

In the cited research, the authors observed that a UV dose of 30–40 mJ/cm^2 would be required to gain 1 log unit of removal while the investigated PAA doses (1–6 mg/L) did not allow any significant removal.

In the present experiment, both disinfection strategies had little effect on *C. perfringens*, leading to a removal always lower than 0.8 and 0.5 log units for UV irradiation and PAA respectively. Taking into account the low removal of *C. perfringens* obtained, additional tests at higher doses of PAA (i.e., 5, 10 and 20 mg/L) were carried out. The results indicated that a PAA dose of 20 mg/L allowed the complete removal of *C. perfringens*. However, such a high dose is not economically sustainable; moreover it would lead to a residual COD concentration in the effluent, which is not compatible with wastewater reuse. Therefore, the biological treatment by SBBGR seems to be the most effective treatment in removing *C. perfringens* with a removal of 2.0 log units.

The biological treatment by SBBGR was characterized by a specific energy consumption of about 0.39 kWh/m^3 of treated wastewater. This value is in line with those reported in the literature for conventional wastewater treatment plants based on activated sludge process (0.45 kWh/m^3) but lower compared to oxidation ditch systems (0.77 kWh/m^3) (Lazarova et al., 2012). Assuming an electricity cost of 12 euro cents per kWh, a cost of 4.68 euro cents per m^3 of wastewater results. The cost of physical or chemical disinfection, however, should be added to this value. Taking into account the operative conditions (i.e., contact time of 1 s) and features (i.e., working volume of 0.5 L and power supply of 40 W) of UV lamp used, a specific energy consumption of only 0.02 kWh (or 0.24 euro cents) per m^3 of wastewater disinfected results. Energy consumption of physical disinfection by UV lamp is lower than the cost of peracetic acid in the chemical disinfection. In fact, on the basis of PAA dosage (i.e., 1 g per m^3 of wastewater) and price (i.e., 20–40 euro/kg), a cost of 2–4 euro cents per m^3 of wastewater disinfected is obtained. These data clearly show that in the case of UV disinfection the relative cost can be considered negligible compared to that of biological treatment by SBBGR.

4. Conclusions

In the present paper, the effectiveness of the SBBGR system and its integration with different disinfection strategies (UV irradiation, PAA addition) to produce an effluent suitable for agricultural use was evaluated.

The main results obtained during the experiment can be summarized as follows:

- Biological treatment by SBBGR was able to produce an effluent with a physical and chemical quality that conforms to the stringent standards required in Italy for agricultural reuse.
- The disinfection performances of the SBBGR were higher than that of conventional municipal wastewater treatment plants for all the investigated microbiological parameters. In particular, the *E. coli* content in the effluent of SBBGR was always below the limit fixed by Italian regulation for discharge into water bodies (5000 CFU/100 mL) but higher than the limit for unrestricted agricultural wastewater reuse (≤10 CFU/100 mL).
- The SBBGR treatment was extremely effective in removing the pathogenic Protozoa *C. parvum* and *G. lamblia*, which are generally highly resistant to conventional biological treatments.
- UV radiation and PAA doses as low as 40 mJ/cm^2 and 1 mg/L respectively were able to reduce *E. coli* content in the final effluent below the limit for agricultural reuse in Italy (i.e., 10 CFU/100 mL).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.11.006.

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