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First Results on the Removal of Emerging Micropollutants from Municipal Centrate by Microalgae

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Abstract **– The results of a first campaign of sampling and analyses of emerging micropollutants in the influent (municipal centrate) and effluent of a pilot MBP raceway are reported. The algal population was chiefly made of *Chlorella* spp. and the pilot worked satisfactorily for the removal of nitrogen. 14 emerging micropollutants were analysed. Average removal efficiencies exceeding 80 % were observed for diclofenac, lamotrigine, ketoprofene, clarithromycin. For such compounds the variability of removal efficiency was also reduced, with respect to the other tested molecules, and was particularly low for diclofenac and lamotrigine. Removal efficiencies over 50 % were measured for azithromycin, metoprolol and irbesartan but with strong variability. Lower removal efficiencies were observed for amisulpride and 5-methylbenzotriazole, while for the remaining compounds the concentrations in the effluent were higher than in the influent.**

Keywords **– Emerging micropollutants; microalgae; removal; pilot raceway; wastewater treatment**

Nomenclature		
Centrate	Liquid phase separated from sewage sludge by centrifugation	–
WWTP	Wastewater Treatment Plant	–
EMP	Emerging micropollutant	–
MBP	Microalgae based process	–
PE	Population Equivalent	–
TSS	Total Suspended Solids	$g\ L^{-1}$
EC	Electric conductivity	$\mu S\ cm^{-1}$
CHP unit	Combined Heat and Power Unit	–
HRT	Hydraulic Retention Time	Days
COD	Chemical Oxygen Demand	$mg\ L^{-1}$
BOD_5	Biochemical Oxygen Demand at 5 days	$mg\ L^{-1}$
Gruppo CAP	Name of the company managing the water service in Milano metropolitan area	

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CAS number	Identifier for chemical substances, based on CAS Registry
HRAP	High Rate Algal Pond

1. INTRODUCTION

The definition of Emerging Micropollutants (EMPs) includes the main problems related to the presence of such compounds in the aquatic environment. They are 'emerging' because their detection is quite recent and their effects on environment and human health are not yet known, especially in terms of dose-response, and they are 'micro' because their environmental concentrations are very low and this involves problems in analysis and quantification as well as in removal from wastewater. That is why for most of them no limit for the discharge in surface water is defined yet, and for some others only guidelines are available. Therefore, further studies are needed and encouraged all over the world.

Pharmaceutical residues are widely represented within EMPs and include a variety of compounds and their degradation products.

Conventional wastewater treatment plants (WWTPs) are designed to remove suspended solids, organic matter (COD and BOD_5), nutrients and potentially hazardous bacteria, but their efficiency in removing EMPs is highly variable and mostly unreliable [1]–[3]. Tertiary treatments are being extensively studied for their efficiency in EMP removal, but a growing interest is laying upon alternative or complementary biological processes.

Among them, microalgal based processes (MBPs) seem very interesting. They are well known for their efficiency in removing nutrients and for the synergy occurring between microalgae and bacteria in open systems, resulting in photo-oxygenation and thus promoting bacterial aerobic metabolism and nitrification with consistent energy savings [4]. In recent years, the ability of MBPs to remove pharmaceutical compounds has been shown. In MBP the removal of EMP can be due to algal adsorption or absorption, to degradation, to bacterial oxidation or simply to chemical or photo-oxidation, as the reactors are designed to maximize the exposure to sun radiation and algal photosynthesis provides large amounts of oxygen, or, more likely, to a combination of such factors [5]. As shown by Abargues *et al.* [6], who studied the removal of some endocrine disruptors in lab-scale reactors, the oversaturation of oxygen, without artificial supply, was such to allow chemical oxidation. In such conditions, the removal was indirectly due to microalgae.

In the study area, i.e. in Northern Italy, weather conditions are not optimal for algae, as in winter temperature and solar radiation are not enough to allow their growth. To overcome this issue, an interesting option is to include the microalgae-based process as a side-stream treatment for the liquid fraction of digestate (defined as centrate when obtained by digestate centrifugation). This is a highly polluted liquid, which is normally sent back from the sludge line to the water line, thus contributing to the load, especially of nitrogen, entering WWTPs. The chief aim of MBP integration in the treatment cycle of WWTPs is the removal of ammonia nitrogen, leading to decrease the ammonia load returned to the main water line, thus reducing the energy demand for nitrification.

Moreover, MBPs can also lead to the removal of other pollutants due to the specific, typical abiotic and biotic conditions, such as the exposure to solar radiation, the high pH-values, and the presence of a diverse microbial community [6]. Being relatively new processes, and due to the limited full scale experiences, few data are available on the ability of MBPs to remove EMPs, and derive mainly from lab-scale [6]–[9] or pilot-scale [10] research, very few of which focusing on the treatment of the liquid fraction of digestate [8].

This work presents a first series of data on the removal of emerging micropollutants in a pilot scale MBP fed on the centrate of a municipal WWTP, along with a synthesis of the overall performances of the process. The reported data on EMPs derive from a sampling campaign, carried out in October 2020, aimed at a preliminary assessment of the EMP load from centrate and on the effect of the MBP on EMP concentrations.

2. METHODS AND METHODOLOGY

2.1. *Pilot plant for MBP*

A raceway pilot plant is installed and working since 2017 [11] within the site of Bresso Niguarda WWTP, operated by Gruppo CAP. It is a conventional municipal WWTP receiving sewage from 220,000 PE, performing mechanical pre-treatments, biological processes (pre-denitrification, biological oxidation and nitrification by activated sludge), filtration and UV disinfection. Sludge is processed by mesophilic (35 °C) anaerobic digestion and thickening. Biogas is sent to two combined heat and power (CHP) units producing 220 and 320 kW, respectively, and solid/liquid separation is carried out by a flocculant-assisted centrifugation. The centrate has the physical-chemical properties reported in Table 1.

TABLE 1. PHYSICAL-CHEMICAL CHARACTERISTICS OF BRESSO-NIGUARDA CENTRATE (MEAN ± STANDARD DEVIATION, $N = 35$)

NH_4-N ($mg\ L^{-1}$)	**NO_3-N ($mg\ L^{-1}$)**	**NO_2-N ($mg\ L^{-1}$)**	**PO_4-P ($mg\ L^{-1}$)**	**TSS ($mg\ L^{-1}$)**	**Optical density 680 nm**	**COD ($mg\ L^{-1}$)**	**EC ($\mu S\ cm^{-1}$)**	**pH**
244 ± 78	0.2 ± 0.2	0.4 ± 0.3	5.7 ± 0.8	83 ± 40	0.1 ± 0.1	112 ± 34	1492 ± 270	8.2 ± 0.3

The pilot scale raceway pond (Fig. 1) has a surface of 5.78 m^2 and a working volume of about 1 m^3. It works in continuous mode and a feeding pump and an overflow allow to keep hydraulic retention time (HRT) at 10 days. CO_2 from the off-gas generated by the CHP unit is provided to support algal growth and to control pH. $NaHCO_3$ is also added, when needed, to increase the buffer capacity of the digestate when intense nitrification occurs.

Fig. 1. Pilot raceway for testing MBP in Bresso-Niguarda WWTP.

The raceway is mixed by a paddle-wheel at a flow velocity of 0.2 m/s. Specific onsite probes allow to monitor continuously dissolved oxygen concentration (Hack Lange, LDO sensor), pH and temperature (Hach Lange, pHD sc Digital Differential pH/ORP Sensors), and turbidity (Hack Lange, Solitax sc Sensors). All the devices are connected to a Programmable Logic Controller (PLC).

2.2. Analyses

Besides direct onsite measurements, lab analyses were carried out twice a week on grab samples to follow microalgal growth and nitrogen removal. Microalgal growth was estimated from Total Suspended Solids (TSS), Optical Density at 680 nm (OD), and cell counts by optical microscope. Algal vitality was monitored by flow cytometry. Chemical Oxygen Demand (COD), nitrogen and phosphorus are analysed by spectrophotometry using Hach Lange test kits (LCK 339, LCK 342, LCK 303, LCK 348, and LCK 314 for nitrate nitrogen, nitrite nitrogen, ammonia nitrogen, phosphate phosphorus and COD, respectively).

The campaign to assess EMP removal was based on eight samplings from the inlet and from the outlet of the raceway (16 samples). Samples were filtered, stored in glass bottles and frozen at −20 °C.

The studied substances are reported in Table 2 and belong to different categories: pharmaceuticals, among which four antibiotics, one industrial compound and one transformation product (TP) derived from the degradation of an antiepileptic drug. They were chosen based on the results of a previous analytical campaign aiming at finding out the most represented EMPs in the centrate from Bresso Niguarda WWTP.

TABLE 2. NAME, CAS NUMBER AND CATEGORY OF THE STUDIED SUBSTANCES

Chemical	**CAS number**	**Category**
Carbamazepine	298-46-4	Pharmaceutical/Antiepileptic
Metoprolol	51384-51-1	Pharmaceutical/Beta blocker
Lamotrigine	84057-84-1	Pharmaceutical/Antiepileptic
Diclofenac	15307-86-5	Pharmaceutical/Anti-inflammatory (NSAID)
Amisulpride	71675-85-9	Pharmaceutical/Anti depressive
Irbesartan	138402-11-6	Pharmaceutical/Cardiovascular drug
5-Methylbenzotriazole	136-85-6	Industrial chemical/Corrosion inhibitor
Propyphenazone	479-92-5	Pharmaceutical/Analgesic-antipyretic
Ofloxacin	82419-36-1	Pharmaceutical/Antibiotic fluoroquinolone
Clarithromycin	81103-11-9	Pharmaceutical/Antibiotic macrolide
Sulfamethoxazole	723-46-6	Pharmaceutical/Antibiotic sulfonamide
Azithromycin	83905-01-5	Pharmaceutical/Antibiotic macrolide
Ketoprofen	22071-15-4	Pharmaceutical/Anti-inflammatory (NSAID)
Gabapentin-lactam	64744-50-9	TP of antiepileptic Gabapentin

Native and isotopically labelled standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO, US) and 100 mg/L stock solutions were prepared in methanol. A final IS working solution of 5 µg/L of 13C6-diclofenac, 50 µg/L of carbamazepine-d10 and ketoprofen-d3, and 500 µg/L of ofloxacin-d3, was prepared diluting with methanol the IS stock solution.

50 µL of IS working solution were spiked in 400 mL of thawed effluent sample and procedural blank samples were prepared with ultrapure water.

Water samples were solid phase extracted on Oasis® HLB cartridges (200 mg/6 mL, Waters, Milford, MA) previously conditioned with 6 mL of methanol followed by 6 mL of

Ultrapure Water (UW). Samples were loaded onto the preconditioned cartridges and extracted at 5–10 mL/min flow rate. When the extraction was completed, the cartridges were washed with 6 mL of UW, air-dried under vacuum for at least 15 min, and then eluted. Otherwise after washing cartridges were stored at −20 °C, dried and eluted after reconditioning at room temperature. Elution was performed with 6 mL of methanol, which was concentrated to 0.5 mL under a stream of nitrogen. The day of the analysis, the extracts were diluted with 0.1 % formic acid (50:50, v/v: 100 µL of sample and 100 µL of aqueous phase) just before injection.

The analyses were carried out by liquid chromatography coupled to mass spectrometry (UHPLC-MS/MS). The chromatographic separation was obtained using methanol and 0.1 % formic acid as eluents, with initial 5 % organic phase and final 100 % organic phase in 10 min. gradient. The flow rate was set to 0.3 mL/min.

The mass spectrometer (TSQ Quantum Access MAX, Thermo Scientific, US) was equipped with heated electrospray ionization (HESI) and operated in positive ion mode. The quantification was performed using selected reaction monitoring (SRM) transitions. The calibration solutions were freshly prepared before each analytical run, by diluting the 100 mg/L stock solutions with 0.1 % formic acid /methanol (50:50, v/v), and preparing eight calibration points between 5 and 1000 µg/L. Limits of detection (LODs) and quantification (LOQs) were estimated as threefold and tenfold the standard deviation of the lowest standard, according to the ISO 6107-2 Standard (ISO 2006). The LODs range was between 0.1 and 6.2 ng/L and the LOQs range was between 0.3 and 20.1 ng/L.

3. RESULTS

Fig. 2 reports the time course of the algal density, quantified in terms of both TSS and OD at 680 nm (the absorbance peak for chlorophyll a), in the period June–November 2020. The two parameters show similar trends, corresponding to a quite steady concentration of microalgae in the system, with maximum levels in the early summer (up to the end of July) and decreasing values in October, in agreement with the general decline in solar radiation and temperature (data not shown). The algal population was dominated by *Chlorella* spp.

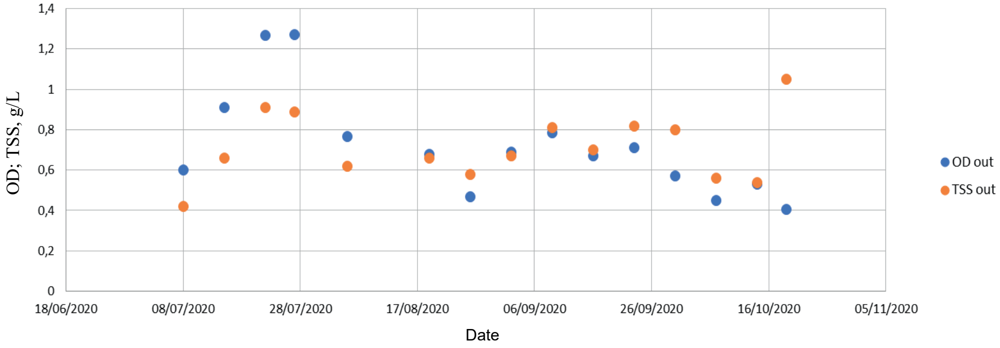

Fig. 2. Time course of microalgal density, expressed as TSS (g L^{-1}) and optical density at 680 nm, during the experimental period

Fig. 3 reports the time course of the main soluble nitrogen forms. While in the influent ammonia nitrogen was the prevailing form, oxidized nitrogen prevailed in the effluent. The overall removal efficiency of the ammonia nitrogen was 83 %. Indeed, thanks to the oxygen provided by photosynthesis, nitrifying bacteria could develop, leading to the production of nitrite and nitrate. Partial nitrification to nitrite was the main process, in agreement with previous findings in similar systems [12], [13]. Nitrification remained the prevailing process leading to ammonia nitrogen removal, while assimilation by algae and bacteria played a minor role.

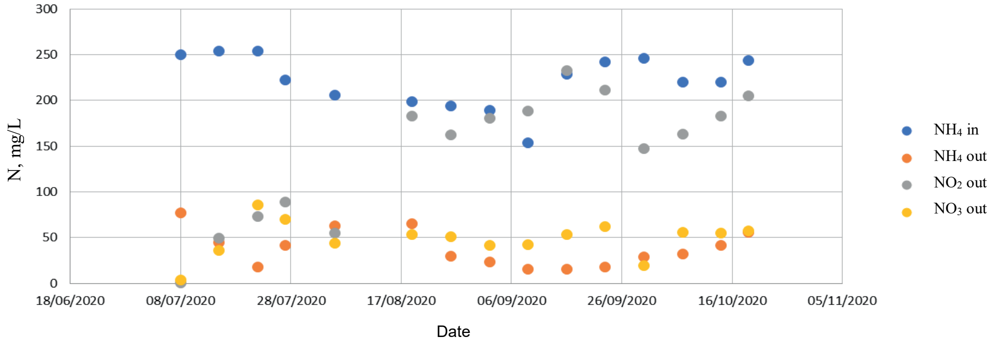

Fig. 3. Concentrations of ammonia nitrogen in the influent and of the different nitrogen forms (ammonia, nitrite and nitrate) in the effluent of the raceway pilot plant during the experimental period.

In some cases, higher concentrations of total nitrogen were observed in the edffluent than in the influent; this difference could be due to water evaporation from the pond surface and to ammonia nitrogen release from the centrate TSS by hydrolysis and ammonification.

The analysis of EMPs gave quite variable results, in agreement with the usual findings in WWTPs [14]–[19]. Table 3 reports the average concentrations obtained in the influent and effluent samples. The presence of EMPs in the centrate derives chiefly from the residues in the activated sludge effluent, due to the only partial removal, but a contribution from the sludge release cannot be excluded.

TABLE 3. INLET AND OUTLET CONCENTRATIONS (OCTOBER 2020) OF THE ANALYSED COMPOUNDS (AVERAGE ± STANDARD DEVIATION)

Chemical	**In, ng/L**	**Out, ng/L**
Carbamazepine	252 ± 23	401 ± 63
Metoprolol	23 ± 19	14 ± 18
Lamotrigine	18 ± 10	1 ± 2
Diclofenac	321 ± 235	5 ± 7
Amisulpride	40 ± 11	27 ± 13
Irbesartan	205 ± 57	94 ± 30
5-methylbenzotriazole	1216 ± 312	1058 ± 219
Propyphenazone	9 ± 2	11 ± 3
Ofloxacin	246 ± 50	300 ± 50
Clarithromycin	3 ± 2	0.5 ± 0.3
Sulfamethoxazole	0.05	0.05
Azithromycin	48 ± 28	16 ± 13
Ketoprofen	151 ± 92	24 ± 25
Gabapentin-lactam	71 ± 10	127 ± 18

Fig. 4 reports the average percent removals observed for the analysed EMPs.

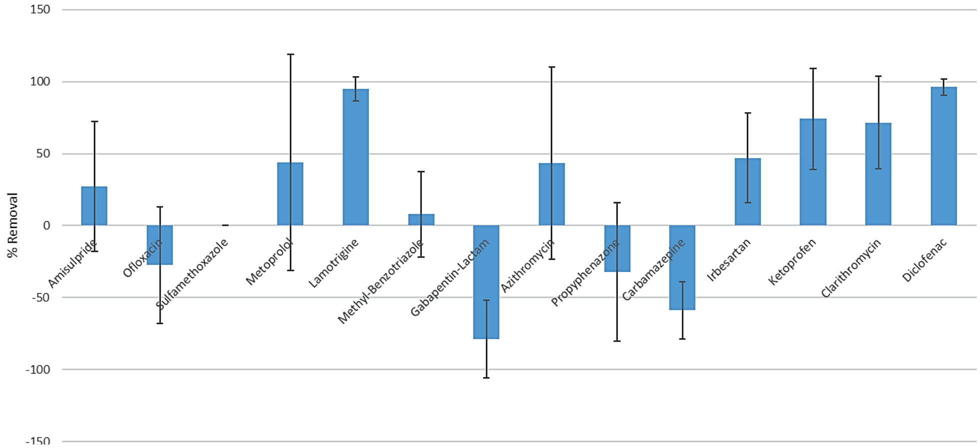

Fig.4. Percent removal of the tested compounds in the MBP pilot raceway (average and standard deviations). Negative values correspond to higher concentrations in the effluent than in the influent.

4. DISCUSSION

The results obtained on EMPs are to be considered as preliminary, due to the low number of samples and to the high variability of the concentrations. Moreover, due to operational problems, the sampling period fell when the microalgal biomass started to decline in density, even if it was still active in nitrogen removal, and analyses were performed on grab samples.

In the present study, diclofenac was one of the most effectively removed compound, as it is in activated sludge processes. Its removal is thus likely to be due to bacterial activity, enhanced by the great oxygen availability. Average removal efficiencies exceeding 80 % in MBP were observed also for lamotrigine and ketoprofen. For such compounds, the variability was also reduced, with respect to the other tested molecules, and was particularly low for diclofenac and lamotrigine. These results agree with the 60–100 % removal efficiency of diclofenac from urine reported by de Wilt et al [7] for *Chlorella sorokiniana* due to the combination of biodegradation and photolysis. The obtained result is particularly interesting for lamotrigine, which is reported not to be removed by activated sludge process [16]. As it often happens with EMPs, for ketoprofen different results are reported in literature. Ismail *et al.* [20] found 20 % removal efficiency in a stirred-tank photobioreactor fed on standard growth medium with high concentrations of ketoprofen (0.5 mM) and other pharmaceutical compounds. Hom-Diaz *et al.* [21], working at pilot scale with a horizontal tubular photobioreactor fed on settled toilet wastewater inoculated with lake microalgae, found very different inlet concentrations in the two experimental periods of the research (472 ± 52 ng L^{-1} in the first and 6729 ± 413 ng L^{-1} in the second one), and removal efficiencies of 36 % and 85 % in the two periods, respectively, with HRT increased from 8 to 12 days. In a pilot scale HRAP fed on wastewater with comparable starting concentrations, Matamoros *et al.* [10] found 50–95 % ketoprofen removal efficiency. The results of both studies are comparable to the ones obtained in the present study.

Removal efficiencies over 50 % were measured for azithromycin (for which a 16 % to 42 % removal is reported for activated sludge processes [16]), metoprolol (for which the reported removal for activated sludge is <40 % [16]), clarithromycin and irbesartan but with strong variability.

The above-mentioned work of Matamoros et al [10] reports for azithromycin 89 % removal, starting from 385±481 ng L^{-1}, with 8 days HRT. For metoprolol removal efficiencies between 60 and 100 %, higher than the ones obtained in the present work, were observed by de Wilt [7], but the experimental conditions were different, being based on lab-scale tests with urine as growth medium.

Escudero *et al.* [22] tested the ability of *Chlamydomonas acidophila* to remove pharmaceutical compounds from a standard growth medium in batch tests (7 days), comparing the removal with microalgae and without them, investigating the effect of abiotic factors on the degradation of such molecules. For clarithromycin they found that the removal efficiency was 50 % and 64 % higher in the presence of microalgae than in their absence, at 8 and 40 μg L^{-1} starting concentration, respectively, while the highest tested concentration (800 μg L^{-1}), inhibited microalgal growth and nutrient uptake. The analysis of the algal cells grown with 8 and 40 μg L^{-1} showed the absence of clarithromycin, demonstrating that bioaccumulation did not occur, while degradation was responsible for the compound removal.

Gentili et al [23] observed 90 % removal efficiency of clarithromycin in wastewater due to microalgae. For Irbesartan very few references exist. Guillossou *et al.* [17] report a null removal in conventional WWTPs and Boix *et al.* [18] report a removal around 25–30 % after 35 days in lab-scale experiment with artificial wastewater inoculated with sewage sludge.

Lower removal efficiencies in the pilot MBP were observed for amisulpride and 5-methylbenzotriazole, while for the remaining compounds the concentrations in the effluent were higher than in the influent. Similar situations had been observed also by other authors who attributed them to either the release from suspended solids or the re-transformations of the metabolites into the parental compounds [19].

The review of existing literature confirms the possible role of microalgae in fostering biological and chemical oxidation, due to the important release of oxygen from photosynthesis in MBP reactors, as well as to the possible photo-oxidation related to light exposure. Adsorption and bioaccumulation can also contribute to decrease EMP concentrations. Enzymatic degradation can occur at intracellular and extracellular level. In fact, many factors interact in the removal of EMPs in MBP reactors and the nature of the compound is important, determining the prevailing removal mechanism. Actually, the present work showed a high removal efficiency for compounds with quite low Kow value: none of the tested EMPs had a value exceeding 4.5, which is reported as the threshold over which bioaccumulation occurs [24]. This could support the hypothesis of degradation by microalgae and/or by the combined action of microalgae and bacteria. When working in continuous, HRT is also important and the long HRT adopted in Bresso-Niguarda pilot plan seems to be adequate to the autumn temperatures of the sampling campaign.

5. CONCLUSION

The MBP, working efficiently in the removal of nitrogen from municipal centrate, showed interesting performances also for EMP removal. Even if the collected data do not allow to draw sound conclusions about the removal efficiencies, the obtained results are encouraging, especially for some compounds, and comparable with the few available literature data, mostly derived from different experimental conditions and in very few cases from centrate fed processes.

For all such reasons, more intensive sampling and analyses need to be performed in order to confirm the obtained results and to correlate them, if it is the case, to the conditions of the microalgal population, which was not optimal in the study period, as well as to the season. Further, it would be interesting to analyse the biomass to assess if bioaccumulation or degradation occurs and/or prevails.

Nonetheless, the research has shown the relevant potential for removing EMPs from the centrate from municipal WWTPs, at the same time as nitrogen.

The advantage of applying MBP consists in the sustainability of the process. As to environmental sustainability the strengths of MBP consist in not involving the addition of chemicals, in the low energy consumption and in the CO_2 absorption. It has been proven that if MBP is integrated in a WWTP the CO_2 requirements of microalgae can be satisfied by the off-gas generated onsite from the CHP, as it was the case in Bresso-Niguarda site, or by CO_2 separated from biogas in methane upgrading process (this option is presently being applied in Bresso-Niguarda WWTP), thus providing a decrease in CO_2 emissions. The economic sustainability can be achieved by the most adequate choice among the several possibilities to valorise the produced biomass [25]. The choice to treat the centrate makes MBP suitable also in non-optimal climate situations, provides bio-oxygenation and helps to decrease the polluting load entering the WWTP, thus enabling better overall performances of the plant, also in the specific case of EMP removal.

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