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Sewage sludge treatment in a thermophilic membrane reactor (TMR): factors affecting foam formation --Manuscript Draft--

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Corresponding Author:	Maria Cristina Collivignarelli, Assistant Professor University of Pavia, Italy ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Pavia, Italy
Corresponding Author's Secondary Institution:	
First Author:	Maria Cristina Collivignarelli, Assistant Professor
First Author Secondary Information:	
Order of Authors:	Maria Cristina Collivignarelli, Assistant Professor Federico Castagnola, Ph.D. Marco Sordi, Ph.D. Giorgio Bertanza, Full Professor
Order of Authors Secondary Information:	
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Abstract:	Foam formation in the excess sludge treatment facilities may represent a critical issue as it could lead to several operative problems and reduce plant performance. Factors affecting foam formation in a thermophilic membrane reactor (TMR), operating with alternate aeration/non aeration cycles and aimed at sewage sludge stabilization and minimization, has been investigated. The experimental study was conducted with a pilot plant (fed daily with thickened sewage sludge) by adjusting the duration of aeration/non aeration alternate cycles. Extracellular polymeric substance (EPS) concentration (and its soluble and bound fractions) has been monitored along with Foaming Power indices. Results highlight that foaming can be correlated to the presence of soluble proteic fraction of EPS. Moreover, EPS production seems to be reduced increasing the duration of the non aeration cycles.
Suggested Reviewers:	Bhargavi Subramanian Illinois Institute of Technology bsubram3@iit.edu Tong Zhang, Associate Professor University of Hong Kong zhangt@hku.hk Steve Petrovski La Trobe University steve.petrovski@latrobe.edu.au
Opposed Reviewers:	
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DEPARTMENT OF CIVIL AND ARCHITECTURAL ENGINEERING
University of Pavia
Via Ferrata, 1 – 27100 Pavia ITALY
Phone. +39 0382 985300 – 985400 – 985450
Fax +39 0382 985589 – 528422 – 985419

Pavia, May 31st, 2016

Dear Editor-in-Chief,

we submit the manuscript titled “Sewage sludge treatment in a thermophilic membrane reactor (TMR): factors affecting foam formation” to *Environmental Science and Pollution Research*.

In this paper factors affecting foam formation in a novel thermophilic membrane biological reactor (TMR), aimed at sludge minimization and operating with alternated aeration(with pure oxygen)/non aeration cycles, are investigated.

The experimental research was performed with a TMR pilot plant (fed daily with thickened sludge) and divided into three different phases (each one characterized by a specific duration of aeration/non aeration alternate cycles). During the experimental phases, extracellular polymeric substances (EPS) concentration (and its soluble and bound fractions) and Foaming Power indices have been monitored.

Results highlight that foaming phenomenon is influenced by the presence of EPS. In effect, direct correlation between foaming power and soluble protein fraction of EPS has been observed. Moreover, the production of EPS seems to be influenced by duration of aeration/non aeration cycles, since an increase in duration of non aeration cycles leads to a progressive reduction of EPS. Therefore, the operative conditions that reduce foam formation have been identified.

All author agree with submission to *Environmental Science and Pollution Research*. This paper is original and unpublished and it is not under consideration for publication by any other journal.

Yours Sincerely.

Maria Cristina Collivignarelli, Federico Castagnola, Marco Sordi
Department of Civil and Architectural Engineering
University of Pavia
Via Ferrata, 1 - 27100 Pavia
Italy

Giorgio Bertanza
Department of Civil, Environmental, Architectural Engineering and Mathematics
University of Brescia
Via Branze 43 – 2 5123 Brescia
Italy

[Click here to view linked References](#)

Sewage sludge treatment in a thermophilic membrane reactor (TMR): factors affecting foam formation

Maria Cristina Collivignarelli^{a*}, Federico Castagnola^b, Marco Sordi^c, Giorgio Bertanza^d

^aDepartment of Civil Engineering and Architecture, University of Pavia, via Ferrata 1, 27100, Pavia, Italy,

mcristina.collivignarelli@unipv.it

^bDepartment of Civil Engineering and Architecture, University of Pavia, via Ferrata 1, 27100, Pavia, Italy,

federico.castagnola@unipv.it

^cDepartment of Civil Engineering and Architecture, University of Pavia, via Ferrata 1, 27100, Pavia, Italy,

marco.sordi@unipv.it

^dDepartment of Civil, Environmental, Architectural Engineering, and Mathematics, University of Brescia, via Branze

43, 25123 Brescia, Italy, giorgio.bertanza@unibs.it

ABSTRACT

Foam formation in the excess sludge treatment facilities may represent a critical issue as it could lead to several operative problems and reduce plant performance. Factors affecting foam formation in a thermophilic membrane reactor (TMR), operating with alternate aeration/non aeration cycles and aimed at sewage sludge stabilization and minimization, has been investigated. The experimental study was conducted with a pilot plant (fed daily with thickened sewage sludge) by adjusting the duration of aeration/non aeration alternate cycles. Extracellular polymeric substance (EPS) concentration (and its soluble and bound fractions) has been monitored along with Foaming Power indices. Results highlight that foaming can be correlated to the presence of soluble proteic fraction of EPS. Moreover, EPS production seems to be reduced increasing the duration of the non aeration cycles.

KEYWORDS

Foaming; EPS; MBR; thermophilic treatment; sludge minimization.

LIST OF ABBREVIATIONS AND ACRONYMS

ATAD	autothermal thermophilic aerobic digestion
COD	chemical oxygen demand
CV	coefficient of variation
EPS	extracellular polymeric substance
EPS _b	bound extracellular polymeric substance
EPS _{b,c}	carbohydrate fraction of bound extracellular polymeric substance
EPS _{b,p}	protein fraction of bound extracellular polymeric substance
FP	foaming power index calculated as consumed sample per litre of supplied air
FP2	foaming power index calculated as foam volume produced per litre of supplied air
MBR	membrane bioreactor
R ²	coefficient of determination
SMP	soluble microbial products

* **Corresponding author:** Phone: +39 0382 985312; Fax: +39 0382 985589; e-mail: mcristina.collivignarelli@unipv.it

1	SMP _c	carbohydrate fraction of soluble microbial products
2	SMP _p	protein fraction of soluble microbial products
3	TMR	thermophilic membrane reactor
4	TN	total nitrogen
5	TP	total phosphorus
6	TS	total solids
7	UF	ultrafiltration
8	UM	unit of measurement
9	VS	volatile solids
10	WWTP	wastewater treatment plant
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1. INTRODUCTION

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18 Foaming in sewage treatment plants is a worldwide occurring problem that affects aeration basins (Guo et al. 2015) and
19 clarifiers (Davenport et al. 2008) operation and performance. The phenomenon is being experimented by 20 - 60% of
20 WWTPs around the world from time to time (Frigon et al. 2006). Moreover, foam formation is one of the most common
21 operating problems in anaerobic sludge digesters (Subramanian and Pagilla 2015) such as in autothermal thermophilic
22 aerobic digesters (ATAD) (Layden et al. 2007).

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25 Foaming issues are widely discussed in the scientific literature as far as conventional activated sludge WWTPs (Xie et
26 al. 2007; Frigon et al. 2006; de los Reyes III and Raskin 2002; Oerther et al. 2001; Pitt and Jenkins 1990) and anaerobic
27 sludge digesters (Subramanian and Pagilla 2015) are concerned. Some studies also investigated foam formation in MBR
28 plants treating municipal wastewater (Nakajima and Mishima 2005; Di Bella et al. 2011; Di Bella and Torregrossa
29 2013). However, as to authors' knowledge, literature on foaming in MBR thermophilic aerobic digesters (a promising
30 technology for sludge minimization which represents an evolution of the - not widespread - thermophilic aerobic
31 digestion: Collivignarelli et al. 2015) is lacking.

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34 Several factors have been identified as possible cause of foam formation: this is indeed a complex phenomenon, which
35 can be ascribed both to microorganism type and/or metabolism and to the presence of synthetic surfactants and other
36 substances such as fats and oils. In case of biological foaming, foam can be due to the massive growth of particular
37 filamentous bacteria (promoted by specific environmental conditions) in the activated sludge and/or to the presence of
38 extracellular polymeric substance (EPS – Nakajima and Mishima 2005; Di Bella et al. 2011; Di Bella and Torregrossa
39 2013) concentration. The production of EPS can be influenced by external factors (presence of toxic substances, air
40 flow regimes, shear rate, redox conditions), substrate type and nutrient shortage (Sheng et al. 2010).

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43 As stated above, the TMR process has been recently proposed for sewage sludge minimization, this aspect being still a
44 pressing demand in municipal WWTPs worldwide (Liu and Tay 2001; Pérez-Elvira et al. 2006; Guo et al. 2013). In this
45 context, the formation of foam in the excess sludge treatment facilities may represent a critical issue as it could lead to
46 operative problems, thus reducing the volatile solids removal yield. In case of open digesters, such as aerobic
47 stabilization reactors, this is even more problematic, due to foam leakage. Finally, filamentous bacteria can be
48 recirculated to the water treatment train, thus maintaining relevant concentration of such organisms in the system and in
49 turn worsening the overall plant performance. Therefore, a better knowledge of the factors affecting foam formation
50 could help in identifying the correct strategies of control and/or prevention.

1 In this paper we report the results of an experimental investigation aimed at studying the foam formation phenomenon
2 in a thermophilic membrane reactor (TMR) with alternate aeration cycles, employed for sewage sludge stabilization and
3 minimization.
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5 **2. MATERIALS AND METHODS**

6 **2.1. Experimental setup and procedures**

7 The thermophilic MBR pilot plant consists of a biological reactor (1 m³ volume) and a side-stream membrane unit
8 (ultrafiltration – 300 kDa, 10 nm) devoted to the liquid/solid separation. The plant is an improved version of the one
9 described in Collivignarelli et al. (2015). In particular, the (pressurized) oxygen is now directly supplied into the sludge
10 recirculation line. Thermophilic conditions (temperature = 55°C) are kept by means of a control system and a heat
11 exchanger mounted on the sludge recirculation line. The plant scheme is reported in Figure 1.
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18 **Fig. 1** Thermophilic MBR pilot plant scheme
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21 The experimentation can be divided into 3 different phases (A, B and C), each one characterized by a specific duration
22 of aeration/non aeration alternate cycles (Table 1).
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25 **Table 1** Duration of aeration and non aeration cycles in the three experimental phases
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28 **2.2. Characteristics of treated sludge**

29 During the entire experimentation (213 days) the pilot plant was fed, daily, with a biological thickened sludge coming
30 from a municipal WWTP (10000 PE, provided with nitrogen removal units), located in Northern Italy. Average
31 chemical and physical characteristics of the sludge are reported in Table 2 (in case of significant variation – higher than
32 10% – the min-max range is also reported). Differences of characteristics all along the experimentation are due to
33 different residence time of the sludge into the thickener where it was sampled. In effect, from phase A to C, an increase
34 in TS and EPS concentration (in particular SMP), together with a decrease of the VS/TS ratio were observed.
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41 **Table 2** Average characteristics of the sludge fed to the TMR during the three experimental phases: where a significant
42 variation ($\pm 10\%$) with respect to the mean value has been recorded, the min-max range is reported
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45 **2.3. Analytical methods laboratory tests**

46 The methods used to measure the concentration of EPS and the procedures to calculate the foaming power of mixed
47 liquor are described below.
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51 **2.3.1. Extracellular Polymeric Substances (EPS)**

52 Extracellular Polymeric Substances (EPS) are produced extracellularly by the microorganisms, mainly under stress. The
53 abbreviation EPS is used as a more general and comprehensive term for different classes of organic macromolecules
54 such as polysaccharides, proteins, nucleic acids, lipids, and other polymeric compounds which have been found to occur
55 in the intracellular space of microbial aggregates, more specifically at or outside the cell surface (Neyens et al. 2004).
56 Furthermore, EPS can be divided into “bound EPS” (EPS_b), which is the fraction contained inside the cell or attached to
57 it, and “soluble EPS” (EPS_s), which is the fraction secreted into the mixed liquor (Rosenberger and Kraume 2003). By
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the way EPS_s can be considered as the generic Soluble Microbial Products (SMP) (as proposed by Laspidou and Rittmann 2002), therefore total EPS can be calculated as:

$$\text{EPS} = \text{EPS}_b + \text{SMP} \quad (1)$$

Heating method was applied to extract EPS from activated sludge. This method was performed according to the procedure described in Le-Clech et al. (2006), Zhang et al. (1999) and Morgan et al. (1990) with some modifications. A portion of activated sludge sample (20 mL) was centrifuged at 5000 rpm for 5 min. The supernatant was filtered (at 0.2 µm) for measuring SMP concentrations while the pellet was re-suspended in 20 mL of distilled water. Re-suspended material was centrifuged again for 10 min and then the centrifuge tube was put in an autoclave at 80 °C for 10 min. After cooling at room temperature it was centrifuged at 7000 rpm for 10 min and the supernatant was filtered (at 0.2 µm) for measuring EPS_b concentrations. Moreover, for both SMP and EPS_b analysis, the concentrations of protein (SMP_p, EPS_{b,p}) and carbohydrate (SMP_c, EPS_{b,c}) were measured by using the Lowry's method (Lowry et al. 1951) and the Anthrone method (Dubois et al. 1956), respectively.

2.3.2. Foaming power tests

The foaming power of mixed liquor was determined according to the procedure proposed by Nakajima and Mishima (2005). This test is performed in a transparent acryl cylinder, stood vertically, with a porous plate on the bottom of it.

The porous plate is connected with an air pump and a flow meter is installed in order to control the airflow.

The foaming power test proposed by Nakajima and Mishima (2005) allows to establish the Foaming Power index (FP) as the amount of sample consumed per litre of supplied air through the equation (2).

$$\text{FP} = (H_0 - H_1) \times S / (Q \times \Delta T) \quad [\text{mL L}_{\text{air}}^{-1}] \quad (2)$$

where:

H₀: the level of the interface between air and sludge at the beginning of the test (cm)

H₁: the level of the interface between sludge ad foam at the end of the test (cm)

S: the area of the cylinder (cm²)

Q: the flow of aeration (L_{air} min⁻¹)

ΔT: the aeration period (min)

In addition, through the equation (3), the Foaming Power index was determined, also, as the amount of foam produced per litre of supplied air. This index was called FP2.

$$\text{FP2} = (H_2 - H_1) \times S / (Q \times \Delta T) \quad [\text{mL L}_{\text{air}}^{-1}] \quad (3)$$

where:

H₂: the level of the interface reached by the foam at the end of the test (cm)

H₁: the level of the interface between sludge and foam at the end of the test (cm)

S: the area of the cylinder (cm²)

Q: the flow of aeration (L_{air} min⁻¹)

ΔT : the aeration period (min)

The Foaming Power indices (FP and FP2) of mixed liquor were monitored starting from the 111th day of experimentation, when we appreciate that the foam issue could be critical, at the same temperature of the biological reactor. Moreover, additional tests were performed changing the operative temperature in order to investigate the behaviour of foam formation. In fact, an increase of temperature may induce modification of the sludge characteristics, such as rheological (Baudez et al. 2013) and/or microbiological properties. For example, higher temperature can involve a decrease of apparent viscosity and surface tension of the sludge with impact on foam drainage (Ganidi et al. 2009). The operative conditions of these tests are summarized in Table 3.

Table 3 Foaming Power tests: operative conditions

3. RESULTS AND DISCUSSION

3.1. EPS monitoring

In Figure 2, the concentration trends of EPS, EPS_b, SMP, together with protein (EPS_{b,p} and SMP_p) and carbohydrate (EPS_{b,c} and SMP_c) components, measured during the overall monitoring campaign, are reported. Chemical analyses show that the EPS concentrations got lower with time (despite an increase of EPS concentration in the fed sludge – Table 2), in correspondence with the progressive increase of the duration of the not aerated cycle (Figure 2a). In effect, starting from 2300 mg L⁻¹ (phase A – 3x4h of aeration and 3x4h of non aeration), the concentrations decreased down to 900 mg L⁻¹ at the beginning of phase B (3x3h of aeration and 3x5h of non aeration) and 500 mg L⁻¹ at the end of the experimentation (phase C – 3x2h of aeration and 3x6h of non aeration).

In the mixed liquor samples, EPS was composed for about 70% of SMP (CV = 16%) (Figure 2a) while, in the fed sludge, SMP ranged from about 1% (phase A) to 30% (phase C). This difference could be due to the cellular lysis occurring during the digestion process with consequent release into the liquid fraction of the cellular content. In effect, as reported by Dai et al. (2013) (regarding anaerobic digestion), the accumulation of SMP in digested sludge is due to the decomposition of floc structures of raw sludge.

As regards the fractionation of EPS_b (which represent the EPS fraction bounded to the cell - Figure 2b) into proteins and carbohydrates, these two components appear to be well balanced during phase A and B. EPS_b protein and carbohydrate concentrations were also characterized by a similar CV (between 22% and 37% for proteins and between 26% and 40% for carbohydrates, respectively). During phase C, the composition of EPS_b changed with a slight increase of protein content, up to 62%. Moreover, the CV coefficient decreased to 6-10%. Finally, SMP (Figure 2c) consisted mainly of proteins in every experimental phase: 56 - 71% (CV = 17%).

Fig. 2 EPS, EPS_b, SMP, concentrations, together with protein (EPS_{b,p} and SMP_p) and carbohydrate (EPS_{b,c} and SMP_c) components, detected in mixed liquor all along each experimental phase (A, B and C)

3.2. Foaming power

The results of the foaming power tests, which were conducted on mixed liquor samples during phases B and C, are shown in Figure 3. The highest values of FP and FP2 were recorded during experimental phase B. Increasing the duration of non aeration cycles, during phase C, both indices showed a decreasing trend with time. In particular, at the end of the experimentation, the FP index was equal to 30 mL of consumed sample per L of supplied air while, the same

index, calculated at the beginning of the phase C, was equal to 160 mL L_{air}⁻¹. The volume of produced foam per L of supplied air (FP2), in phase C, passed from 1200 mL L_{air}⁻¹ (at the beginning of the phase) to 550 mL L_{air}⁻¹ (at the end of the phase). Therefore, the increased duration of non aeration cycles led to an overall decrease of the foam potential.

Fig. 3 Foaming power (FP and FP2 indices) evolution during the experimentation

The influence of temperature on foam formation potential (FP and FP2 indices) was also investigated. In Figure 4 the results of tests conducted at different temperatures are reported: they are normalized with respect to values calculated at 25°C. The results show that the higher the temperature the higher the volume of produced foam (Figures 4c and 4d). This effect is particularly evident in phase C (Figure 4d) where, at the highest temperature (55°C), the average increase in FP2 indices (hence in foam volume) was about 18% with respect to the FP2 indices calculated at 25°C. However, as regards the consumed volume during foam formation (FP index - Figure 4a and 4b), no significant increases were observed. In effect, the overall increase in FP indices with temperature was below 2% (with respect to FP values at 25°C) during phase B and below 8% for phase C.

Fig. 4 Correlation between temperature and Foaming Power (FP and FP2) indices of mixed liquor for different days of experimentation and operative phases

3.3. Correlation between EPS and Foaming Power

The EPS have a significant influence on chemical-physical properties of the microbiological aggregates (surface charge, flocculation, settlability, etc.) (Sheng et al. 2010). As reported in the introduction, some studies have highlighted that the foam formation in biological sewage sludge could be influenced by the presences of EPS (Nakajima and Mishima 2005; Di Bella et al. 2011; Di Bella and Torregrossa 2013).

In Figure 5 and Figure 6 measured power indices (FP and FP2) and related EPS concentrations (and their fractions: EPS_b, SMP, etc.) are reported. From the obtained results it is possible to observe that the FP index presents a good correlation with the soluble fraction of EPS (SMP - Figure 5b), in particular with the protein fraction (Figure 5e). Both fractions of proteins (Figure 5f) and carbohydrates (Figure 5i) bound to cell, instead, show no correlation with the same foaming power index (lowest R² values). A weak correlation was also observed between FP2 index and SMP fractions (Figures 6b, 6e and 6h).

Experimental results show that the foam formation may be favoured by the presence of SMP and, in particular, by the presence of soluble proteins. These findings are apparently in contrast with those obtained by Di Bella et al. (2011) and Di Bella and Torregrossa (2013), according to which the presence of bound EPS is in correlation with the FP index. Actually this difference can be explained with the different nature of the tested sludge. In effect, Di Bella et al. (2011) and Di Bella and Torregrossa (2013) worked with the sludge taken from mesophilic MBR pilot plants treating urban wastewater, while in our case the feeding substrate was excess sludge and the process was operated under thermophilic temperatures and alternate aeration cycles.

Fig. 5 Correlation between FP and EPS, bound EPS, SMP and their fractions (proteins and carbohydrates)

Fig. 6 Correlations between FP2 and EPS, bound EPS, SMP and their fractions (proteins and carbohydrates)

4. CONCLUSIONS

A TMR pilot plant was monitored in order to study possible factors affecting foam formation. The main findings of the research are the following:

- the studied process causes an appreciable hydrolyzation of the floc structure and cell-lysis, observable with an increase of SMP concentrations with respect to total EPS.
- The operating conditions (aeration/non aeration cycles) have an evident effect on EPS content of the sludge; in particular, an increase in duration of non aeration cycles leads to a decrease of EPS concentration (hence of SMP and EPS_b).
- The duration of aeration/non aeration cycles also influences the presence of foam. In effect, foaming power decreases with an increase of duration of non aeration cycles.
- A good correlation between foaming power and protein fraction of SMP has been observed.

In short, in the TMR process, the foam formation is correlated to the presence of EPS, in particular the soluble proteic fraction (SMP_p). The presence of these substances depends on the duration of aeration/non aeration cycles, an increase in duration of non aeration cycles leading to a progressive reduction of EPS. In light of this, in order to reduce foam, phase C (3x2h of aeration and 3x6h of non aeration) was identified as the best operative condition.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that there is no conflict of interest regarding the publication of this paper.

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.

REFERENCES

- Baudez J-C, Gupta RK, Eshtiaghi N, Slatter P (2013) The viscoelastic behaviour of raw and anaerobic digested sludge: Strong similarities with soft-glassy materials. *Water Res* 47(1):173-180. doi: 10.1016/j.watres.2012.09.048
- Collivignarelli MC, Castagnola F, Sordi M, Bertanza G (2015) Treatment of sewage sludge in a thermophilic membrane reactor (TMR) with alternate aeration cycles. *J Environ Manage* 162:132-138. doi: 10.1016/j.jenvman.2015.07.031
- Dai X, Luo F, Dai L, Dong B (2013) Degradation of Extracellular Polymeric Substances (EPS) in Anaerobic Digestion of Dewatered Sludge. *Procedia Environmental Sciences* 18:515-521. doi: 10.1016/j.proenv.2013.04.069
- Davenport RJ, Pickering RL, Goodhead AK, Curtis TP (2008) A universal threshold concept for hydrophobic mycolata in activated sludge foaming. *Water Res* 42(13):3446-3454. doi: 10.1016/j.watres.2008.02.033
- de los Reyes III FL, Raskin L (2002) Role of filamentous microorganisms in activated sludge foaming: relationship of mycolata levels to foaming initiation and stability. *Water Res* 36(2):445-459. doi: 10.1016/S0043-1354(01)00227-5

- 1 Di Bella G, Torregrossa M (2013) Foaming in membrane bioreactors: Identification of the causes. *J Environ Manage*
2 128:453-461. doi: 10.1016/j.jenvman.2013.05.036
3
- 4 Di Bella G, Torregrossa M, Viviani G (2011) The role of EPS concentration in MBR foaming: Analysis of a submerged
5 pilot plant. *Bioresource Technol* 102(2):1628-1635. doi: 10.1016/j.biortech.2010.09.028
6
7
- 8 Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination for sugars and
9 related substances. *Anal Chem* 28(3):350-356. doi: 10.1021/ac60111a017
10
11
- 12 Frigon D, Guthrie RM, Bachman GT, Royer J, Bailey B, Raskin L (2006) Long-term analysis of a full-scale activated
13 sludge wastewater treatment system exhibiting seasonal biological foaming. *Water Res* 40(5):990-1008. doi:
14 10.1016/j.watres.2005.12.015
15
16
- 17 Ganidi N, Tyrrel S, Cartmell E (2009) Anaerobic digestion foaming causes – A review. *Bioresource Technol*
18 100(23):5546-5554. doi: 10.1016/j.biortech.2009.06.024
19
20
- 21 Guo F, Wang Z-P, Yu K, Zang T (2015) Detailed investigation of the microbial community in foaming activated sludge
22 reveals novel foam formers. *Scientific Reports* 5:7637. doi: 10.1038/srep07637
23
24
- 25 Guo W-Q, Yang S-S, Xiang W-S, Wang X-J, Ren N-Q (2013) Minimization of excess sludge production by in-situ
26 activated sludge treatment processes – A comprehensive review. *Biotechnol Adv* 31(8):1386-1396. doi:
27 10.1016/j.biotechadv.2013.06.003
28
29
- 30 Laspidou CS, Rittmann BE (2002) A unified theory for extracellular polymeric substances, soluble microbial products,
31 and active and inert biomass. *Water Res* 36(11):2711-2720. doi: 10.1016/S0043-1354(01)00413-4
32
33
- 34 Layden MN, Mavinic DC, Kelly HG, Moles R (2007) Autothermal thermophilic aerobic digestion (ATAD) – Part I:
35 Review of origins, design, and process operation. *J Environ Eng Sci* 6(6):665-678. doi: 10.1139/S07-015
36
37
- 38 Le-Clech P, Chen V, Fane TAG (2006) Fouling in membrane bioreactors used in wastewater treatment. *J Membrane*
39 *Sci* 284(1-2):17-53. doi: 10.1016/j.memsci.2006.08.019
40
41
- 42 Liu Y, Tay J-H (2001) Strategy for minimization of excess sludge production from the activated sludge process.
43 *Biotechnol Adv* 19(2):97-107. doi: 10.1016/S0734-9750(00)00066-5
44
45
- 46 Lowry OH, Rosenbrough NJ, Farr AI, Randall RJ (1951). Protein measurement with Folin phenol reagent. *J Biol Chem*
47 193:265-275.
48
49
- 50 Morgan JW, Forster CF, Evison L (1990) A comparative study of the nature of biopolymers extracted from anaerobic
51 and activated sludges. *Water Res* 24(6):743-750. doi: 10.1016/0043-1354(90)90030-A
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 Nakajima J, Mishima I (2005) Measurement of foam quality of activated sludge in MBR. *Acta hydroch hydrob*
2 33(3):232–239. doi: 10.1002/aheh.200400575

3
4 Neyens E, Baeyens J, Dewil R, De heyder B (2004) Advanced sludge treatment affects extracellular polymeric
5 substances to improve activated sludge dewatering. *J Hazard Mater* 106(2-3):83-92. doi: 10.1016/j.jhazmat.2003.11.014
6
7

8
9 Oerther DB, de los Reyes III FL, de los Reyes MF, Raskin L (2001) Quantifying filamentous microorganisms in
10 activated sludge before, during, and after an incident of foaming by oligonucleotide probe hybridizations and antibody
11 staining. *Water Res* 35(14):3325-3336. doi: 10.1016/S0043-1354(01)00057-4
12
13

14
15 Pérez-Elvira SI, Nieto Diez P, Fdz-Polanco F (2006) Sludge minimisation technologies. *Reviews in Environmental*
16 *Science and Bio/Technology* 5(4):375-398. doi: 10.1007/s11157-005-5728-9
17
18

19 Pitt P, Jenkins D (1990) Causes and control of *Nocardia* in activated sludge. *Res J Water Pollut C* 62(2):143-150.
20
21

22 Rosenberger S, Kraume M (2003) Filterability of activated sludge in membrane bioreactors. *Desalination* 151(2):195-
23 200. doi: 10.1016/S0011-9164(02)00998-0
24
25

26
27 Sheng G-P, Yu H-Q, Li X-Y (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological
28 wastewater treatment systems: A review. *Biotechnol Adv* 28(6):882-894. doi: 10.1016/j.biotechadv.2010.08.001
29
30

31 Subramanian B, Pagilla KR (2015) Mechanisms of foam formation in anaerobic digesters. *Colloid Surface B* 126:621-
32 630. doi: 10.1016/j.colsurfb.2014.11.032
33
34

35
36 Xie B, Dai X-C, Xu Y-T (2007) Cause and pre-alarm control of bulking and foaming by *Microthrix parvicella* - A case
37 study in triple oxidation ditch at a wastewater treatment plant. *J Hazard Mater* 143(1-2):184-191. doi:
38 10.1016/j.jhazmat.2006.09.006
39
40

41
42 Zhang X, Bishop PL, Kinkle BK (1999) Comparison of extraction methods for quantifying extracellular polymers in
43 biofilms. *Water Sci Technol* 39(7):211-218. doi: 10.1016/S0273-1223(99)00170-5
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Phase	First – last day	Duration [d]	Number per day and duration [h] of:	
			non aeration	aeration
A	0 – 58	58	3 x 4h =12	3 x 4h =12
B	59 – 143	85	3 x 5h =15	3 x 3h = 9
C	144 – 213	70	3 x 6h =18	3 x 2h = 6

Table 1 Duration of aeration and non aeration cycles in the three experimental phases

Parameter	UM	Phase A	Phase B	Phase C
COD	[mg L ⁻¹]	23850	25000	26350 - 40650
TN	[mg L ⁻¹]	1120	1230	1140 - 2030
TP	[mg L ⁻¹]	460	460 - 690	535 - 650
SMP	[mg L ⁻¹]	5.5	8.0 - 140	110 - 430
SMP _p	[mg L ⁻¹]	2.5	1.0 - 2.2	60 - 300
SMP _c	[mg L ⁻¹]	3.0	5.9 - 140	48 - 130
EPS _b	[mg L ⁻¹]	488	660 - 1180	220 - 1215
EPS _{b,p}	[mg L ⁻¹]	480	600 - 630	80 - 1000
EPS _{b,c}	[mg L ⁻¹]	8	62 - 560	30 - 215
EPS (SMP + EPS _b)	[mg L ⁻¹]	495	670 - 1320	330 - 1640
TS	[g L ⁻¹]	27.8	30.2	29.8 - 52.0
VS	[g L ⁻¹]	15.3	16.2	15.9 - 25.2
VS/TS	[%]	55.0	53.9	50.6

Table 2 Average characteristics of the sludge fed to the TMR during the three experimental phases: where a significant variation ($\pm 10\%$) with respect to the mean value has been recorded, the min-max range is reported

Test	Sample	Volume [mL]	Temperature [°C]	S [cm ²]	Q [L _{air} min ⁻¹]	ΔT [min]
Monitoring	Raw Mixed liquor	100	55	9.62	1.0	0.5
Additional tests	Raw Mixed liquor	100	25, 35, 48, 55	9.62	1.0	0.5

Table 3 Foaming Power tests: operative conditions

[Click here to view linked References](#)

Fig. 1 Thermophilic MBR pilot plant scheme

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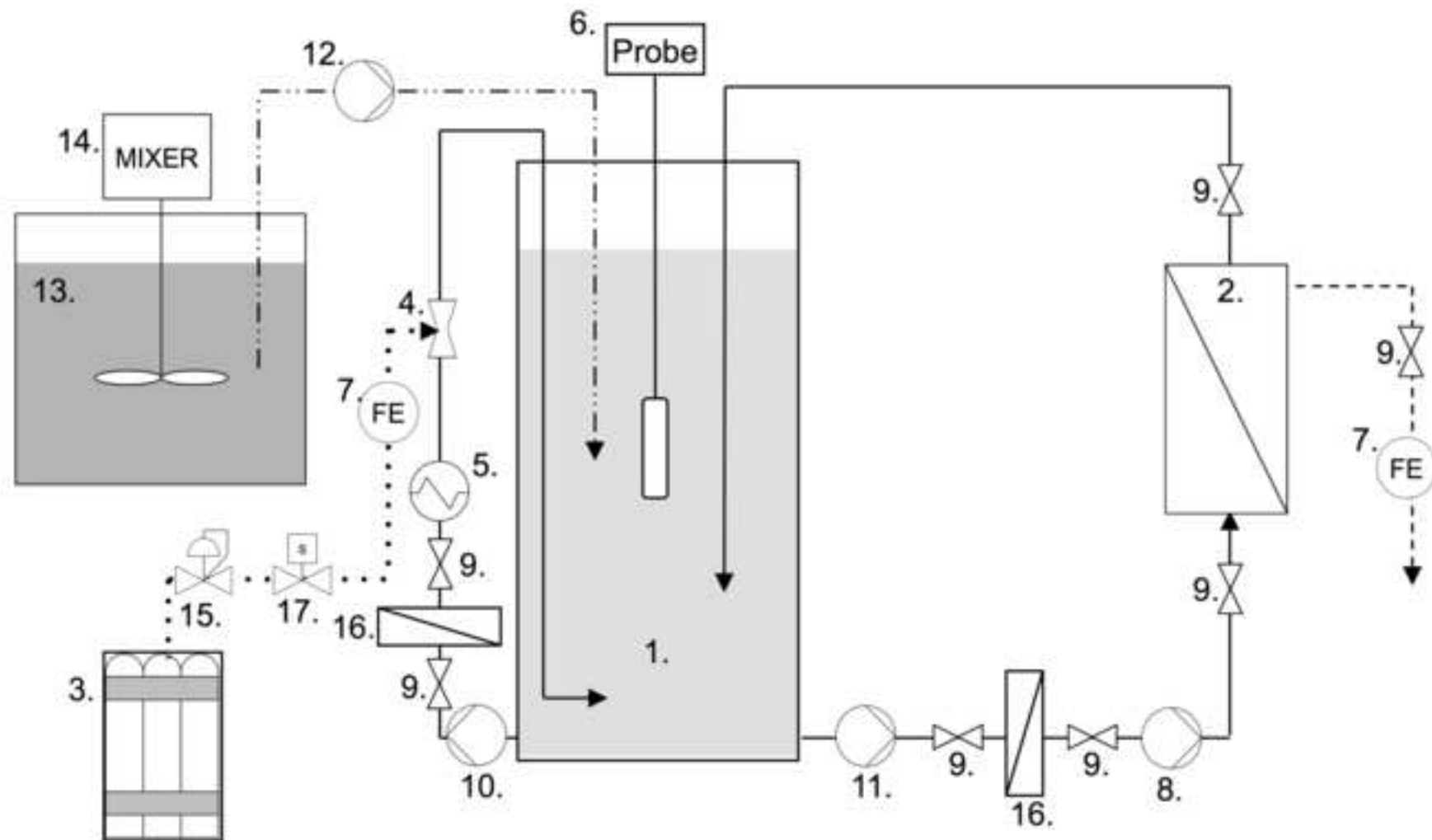
Fig. 2 EPS, EPS_b, SMP, concentrations, together with protein (EPS_{b,p} and SMP_p) and carbohydrate (EPS_{b,c} and SMP_c) components, detected the in mixed liquor all along each experimental phase (A, B and C)

Fig. 3 Foaming power (FP and FP2 indices) evolution during the experimentation

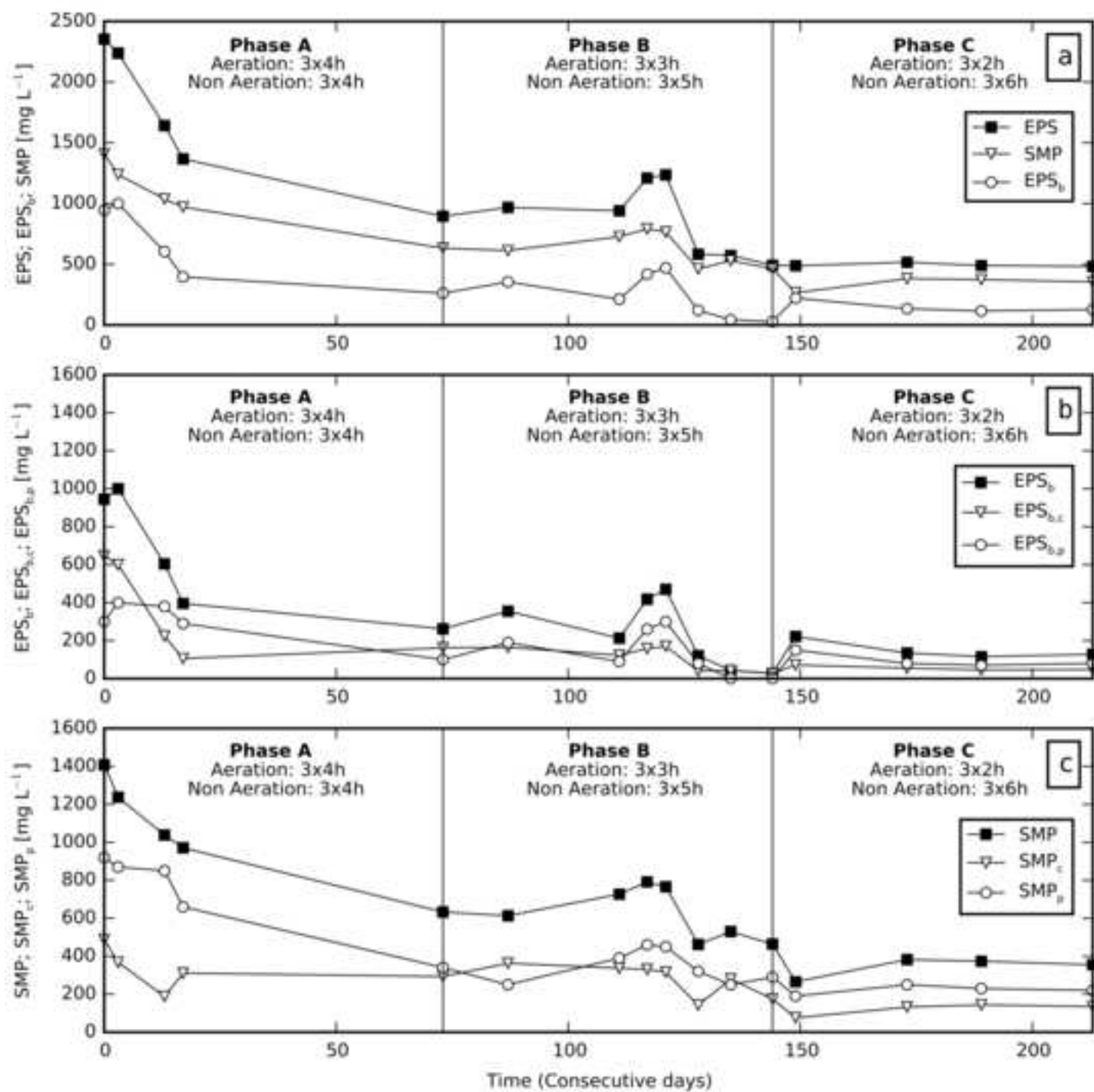
Fig. 4 Correlation between temperature and Foaming Power (FP and FP2) indices of mixed liquor for different days of experimentation and operative phases

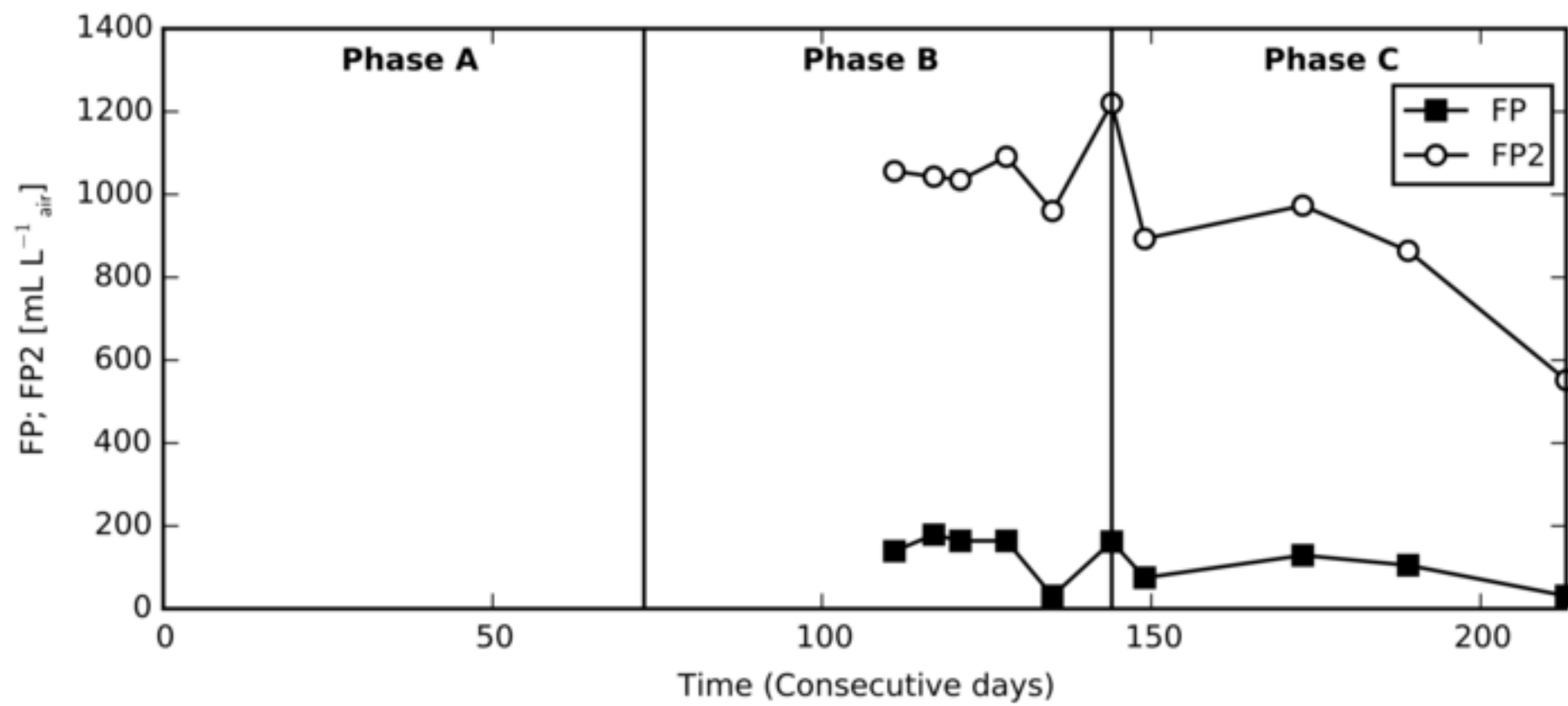
Fig. 5 Correlation between FP and EPS, bound EPS, SMP and their fractions (proteins and carbohydrates)

Fig. 6 Correlations between FP2 and EPS, bound EPS, SMP and their fractions (proteins and carbohydrates)



1.	Biological reactor (1 m ³)	9.	Valve	17.	Electrovalve
2.	UF membrane vessel	10.	Recirculation pump		
3.	O ₂ tanks	11.	Pump		
4.	O ₂ mixer	12.	Feeding pump	-----	Substrate
5.	Heat-exchanger	13.	Substrate tank (1 m ³)	—————	Sludge
6.	O ₂ and temperature probe	14.	Mixer	- - - - -	Permeate
7.	Flowmeter	15.	Pressure reducer	O ₂
8.	Pressurizing pump	16.	Coarse filter (1.5 mm)		





Consecutive experimental days

Phase B: \square 111th ∇ 117th \circ 121st \diamond 128th \triangle 135th
 Phase C: \blacksquare 144th \blacktriangledown 149th \bullet 173rd \blacklozenge 189th \blacktriangle 213rd

