

Growth of microalgal biomass on supernatant from biosolid dewatering

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INTRODUCTION

Microalgae cultivation is nowadays being considered as an attractive alternative to produce renewable energy in an ecologically, socially and economically sound way ([De Schampheleire & Verstraete 2000](#)). With respect to conventional energy crops, microalgae cultivation offers substantial advantages, namely: the land-specific productivity, the lower land-quality requirements which reduce the competition with food crops for arable land use, both the lesser quality and quantity of water and nutrients demanded, and the expected decreased footprint upon the environment. Moreover, microalgae grow faster than any other phototrophic organism and perform photosynthesis 10–50 times more efficiently than plants ([Li *et al.* 2000](#)). These recognized advantages have motivated a recent renewed interest in both efficient microalgae cultivation techniques and their exploitation for ethanol, biodiesel, biomethane or biohydrogen production, or in a combined multi-step 'bio-refinery' concept for comprehensive conversion of solar radiation into biofuels ([Mata *et al.* 2010](#)).

Besides light, microalgae need nutrients (mainly N and P) and CO₂ to grow. Nutrients are contained in excess in most liquid wastes, such as domestic wastewater. Those streams need dedicated treatments to reduce their nutrient loads to be safely discharged. The integration of microalgae cultivation within a typical wastewater treatment plant (WWTP) scheme (activated sludge with nitrogen removal, anaerobic digestion of primary and excess sludge and combined heat and power (CHP) generation for biogas valorization) sounds interesting since it may take advantage of the availability of nutrient-rich streams (the supernatant from anaerobic digestion) and of a CO₂-rich stream (the CHP off-gas). Moreover, N removal would be a further positive effect deriving from a process designed to improve the production of energy from anaerobic digestion. Previous studies have shown that microalgae are able to capture CO₂ from CO₂-rich streams such as flue and flaring gases with CO₂ contents in the range 5–15% ([Hsueh *et al.* 2007](#)). Existing facilities may be exploited, such as solid/liquid separation

units for microalgae thickening and the anaerobic digester for microalgae biomethanization. This sounds especially profitable for those many digesters in WWTPs originally designed for chemical oxygen demand (COD) removal that have then been upgraded in order to remove nitrogen, thus working on sludge of greater age. It is known that in such conditions sludge degradability is low so that specific biogas production drops and the existing digesters are underloaded (Bolzonella *et al.* 2002). In Figure 1, the optimal location of the photobioreactor for microalgae cultivation within a conventional WWTP is sketched.

We report here the results of experiments to test the feasibility growing microalgae on the centrate from belt-press dewatering of digested anaerobic sludge. Preliminary results are also presented on the anaerobic biodegradability of the cultivated algal suspension in biochemical methane potential tests and on the settleability of the algal suspension after mixing with the activated sludge.

MATERIALS AND METHODS

Microalgae inocula

Microalgae inocula (*Botryococcus braunii* and *Chlorella* sp.) were purchased from EPSAG (Göttingen University,

Germany). A sample of *Scenedesmus obliquus* was kindly supplied by INRA-LBE (Narbonne, France).

Reactors and equipment

Three types of reactors were used (Figure 2): (a) glass Erlenmeyers (0.5–3 L in volume); (b) a PVC reactor made of four rectangular columns with a maximum working volume of 5.5 L each; (c) a Plexiglas column of 12 cm diameter (11 L volume).

Temperature was kept at room values (20–22 °C) and mixing was provided by gas bubbling.

Light was provided by six fluorescent OSRAM, FLORA, 18 W lamps supplying a photosynthetic active radiation (PAR) of $230 \mu\text{mol m}^{-2} \text{s}^{-1}$ on 16 h light/8 h dark cycles.

Some tests were conducted using air as the sole CO₂ source, while for some others CO₂ was supplied by pure gas injection.

Microscopic observations were performed with an optical microscope (Nikon).

Analytical methods

Analyses were carried out according to the Standard Methods for ammonia, nitrate and total nitrogen, total phosphorus, COD and total suspended solids (TSS). Algal concentration was measured based on TSS values.

Biochemical methane production (BMP) and settling tests

BMP tests were performed in duplicate using a commercial laboratory instrument (AMTPS, Bioprocess control, Sweden). This was a volumetric device consisting of 15 gas-tight glass bottles (500 mL of working volume) located in a water bath at 35 ± 0.5 °C. Each bottle was continuously mixed with a mechanical rotary stirrer. The biogas produced

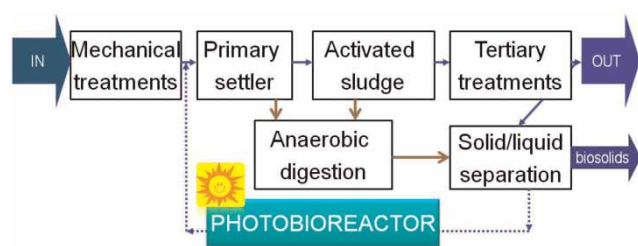


Figure 1 | Integration of microalgae cultivation within a conventional wastewater treatment plant.

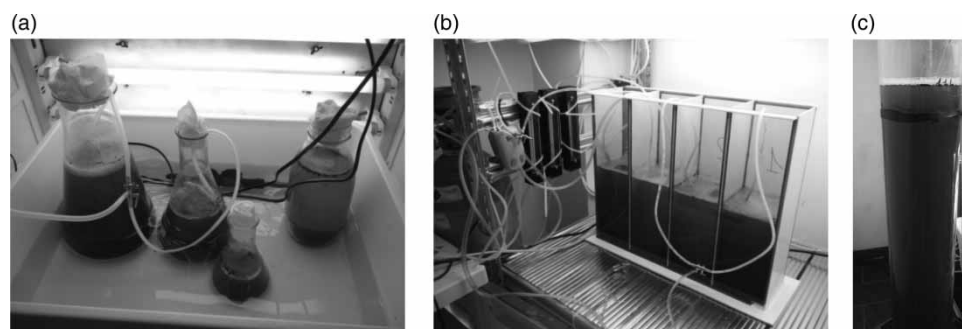


Figure 2 | Reactors for microalgae cultivations: (a) conventional glass Erlenmeyers; (b) PVC installation made of four parallel reactors (R1–4); (c) Plexiglas column (C).

passed through a NaOH solution (3 M), for CO₂ absorption. Methane flowed through a liquid-displacement automated measuring unit with a resolution of 11–13 mL. A data acquisition system allowed flow-rate data to be recorded continuously. The anaerobic inoculum used for BMP tests was obtained from a digester fed on waste activated sludge. The testing protocol was set according to Angelidaki *et al.* (2009). Relevant testing conditions were: a temperature of 35 ± 0.5 °C, an inoculum to substrate ratio of 10 gVS/gVS, and a digestion time of 27 d (days). Tests were performed in duplicate and a blank bottle for the assessment of aspecific methane production was included.

Settling tests were performed on samples obtained by blending the algal suspension with various proportions (0, 30%, 70%, 100% v/v) of activated sludge. The suspension was mixed by a Jar test apparatus for 30 sec at 150 rpm and for a further 10 min at 45 rpm. Then, the Sludge Volume Index (SVI) and the residual suspended solids were measured.

RESULTS AND DISCUSSION

Preliminary batch tests

In these tests, algal inocula were cultivated on a mineral medium (EPSAG Basal Medium ES) or on a mixture of the final effluent (after sand filtration) and centrate from the belt-press. Table 1 reports their analytical characterization.

Nutrient concentrations were adjusted to 50 mg N/L and 4 mg P/L. Suspended solids, ammonium and nitrate were measured through time until N-limiting conditions were achieved. The average specific growth rate (μ) was estimated by using the TSS data (X), from the average slope of TSS growth against time ($\Delta X/\Delta t$), as follows

$$\mu = \frac{\Delta X}{X \cdot \Delta t}$$

Table 1 | Average composition of the final effluent and of the centrate from sludge dewatering used as substrate for algal culture

Parameter	Final effluent	Centrate from sludge dewatering
NO ₃ -N (mg/L)	9 ± 2.0	10 ± 3.9
NH ₄ -N (mg/L)	4 ± 5.5	252 ± 49
TSS (mg/L)	8 ± 5.1	120 ± 50
P (mg/L)	1.67 ± 0.30	8 ± 4
COD (mg/L)	51 ± 13.8	470 ± 131

Average values of μ (d⁻¹) for the available algal inocula grown on a mineral medium only were: 0.146 ± 0.019, 0.201 ± 0.008, 0.172 ± 0.017 for *Chlorella*, *Botryococcus*, and *Scenedesmus*, respectively. Growth rates were found to be similar among the tested species. Therefore, a mixed sample of the above-mentioned microalgae was later used to assess μ when microalgae were grown on a mixture of final effluent (SE) and centrate (C); pure CO₂ was here bubbled for mixing at a low flow rate (10 L/h). Comparing the results obtained with different SE:C ratios (in 5.5 L PVC reactors) and those obtained on a mineral medium (in 0.5–3 L glass Erlenmeyers), lower but still significant growth rates ($\mu = 0.072 \pm 0.004$ d⁻¹, 0.078 ± 0.006 d⁻¹ for SE:C = 10:1 and SE:C = 6:1 respectively) for microalgae grown on a reclaimed water stream were obtained.

Cultivation of microalgae on centrate

These tests were conducted in parallel by using three out of the four available PVC reactors (referred to as R1, R2 and R3) and the Plexiglas column (referred to as C). Feeding and discharge was performed manually 2–3 times per week. Mixing and carbon supply was provided by bubbling a gas mixture made of air (90%) and CO₂ (10%) at a rate of 0.06L/min. The feed was made by blending the final WWTP effluent with the centrate from sludge dewatering (see Table 1) and flow rate was adjusted to maintain the hydraulic retention time (HRT) at 10 d. The percentage of centrate in the feed was increased in three steps, which identified the experimental phases. Relevant operational conditions of each experimental phase are summarized in Table 2. Reactors were monitored by measuring ammonium, nitrate, pH, and suspended solids on the algal suspension.

In Figure 3, the trends of influent and effluent nitrogen (N_{in} and N_{out}) and suspended solids (SS_{in} and SS_{out}) during the course of the experimentation are plotted, while their average values for each experimental phase are summarized in Table 3. It can be seen that the effluent nitrogen concentration increased with the increase in the nitrogen load. Similar results were obtained in all reactors with a slightly

Table 2 | Average values of the main operational parameters of algal cultivation on centrate for PVC reactors (R1–4) and Plexiglas column (C)

Phase	Fraction of centrate in feed, %	N _{in} (mg/L)	Nitrate in feed (mg/L)	SS _{in} (g/L)
1	10–15	50 ± 2	9 ± 2	0.12 ± 0.00
2	20–25	72 ± 3	8 ± 2	0.15 ± 0.01
3	100	271 ± 54	13 ± 2	0.12 ± 0.05

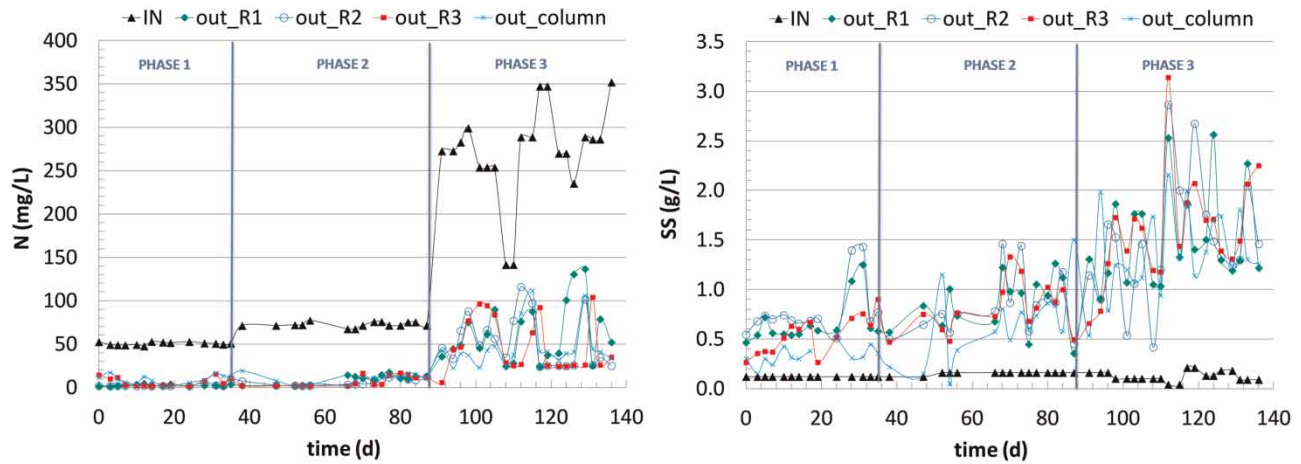


Figure 3 | Total N (left) and suspended solid (right) concentrations at the inlet and at the outlet of the column and of the PVC reactors (R1, R2, and R3) during the course of the experiment.

Table 3 | Average efficiency parameters of algal cultivation on centrate for PVC reactors (R1–4) and Plexiglas column (C)

Phase	Reactor	N _{out} (mg/L)	SS _{out} (g/L)	pH	Average τ_{1N}	$\Delta SS/\Delta N$
1	R1	2 ± 1	0.66 ± 0.22	6.9 ± 1.0	0.95 ± 0.02	11.3 ± 4.6
	R2	3 ± 2	0.78 ± 0.28	6.9 ± 1.0	0.95 ± 0.04	11.9 ± 6.1
	R3	6 ± 5	0.54 ± 0.19	6.9 ± 1.1	0.87 ± 0.10	9.7 ± 4.9
	C	9 ± 5	0.34 ± 0.09	8.0 ± 1.0	0.82 ± 0.10	5.8 ± 1.6
2	R1	9 ± 5	0.85 ± 0.28	8.0 ± 0.8	0.87 ± 0.08	11.2 ± 4.7
	R2	6 ± 4	0.84 ± 0.31	8.0 ± 0.8	0.91 ± 0.06	10.4 ± 4.6
	R3	7 ± 6	0.81 ± 0.26	8.0 ± 0.8	0.90 ± 0.08	10.3 ± 4.6
	C	10 ± 5	0.65 ± 0.38	8.4 ± 0.9	0.86 ± 0.07	8.7 ± 3.8
3	R1	62 ± 34	1.52 ± 0.49	7.2 ± 1.2	0.77 ± 0.13	7.2 ± 3.1
	R2	52 ± 30	1.50 ± 0.61	7.1 ± 0.6	0.80 ± 0.13	6.7 ± 3.9
	R3	49 ± 31	1.60 ± 0.54	7.2 ± 1.2	0.82 ± 0.11	6.9 ± 2.4
	C	47 ± 25	1.38 ± 0.43	7.8 ± 0.5	0.82 ± 0.09	6.0 ± 2.7

higher effluent N concentration in the column during phases 1 and 2. The resulting N removal efficiency (η_N) is also reported in Table 3 and it is generally included between 77 and 95%, the lower values being registered during phase 3.

As for algal growth, suspended solids were measured as an indication of the concentration of microalgae. As shown in Figure 3, suspended solid concentration increased in all reactors with the increase in the influent N load, and a similar trend was observed for all four reactors. Finally, the $\Delta SS/\Delta N$ value was computed as follows:

$$\Delta SS/\Delta N = \frac{(SS_{out} - SS_{in})}{(N_{in} - N_{out})}$$

As shown in Table 3, the mass of suspended solids produced per mass of nitrogen utilized remained between 6 and

11 (gSS/gN), the high dispersion being caused by the variability in effluent SS concentrations. Moreover, the $\Delta SS/\Delta N$ ratio tended to decrease in phase 3, confirming a less effective conversion of the available nitrogen into new algal cells.

Finally, by making a steady-state SS mass balance, the average specific growth rate can be computed as:

$$\mu = \frac{(SS_{out} - SS_{in})}{HRT \times SS_{out}}$$

with the result of 0.057 ± 0.001 , 0.044 ± 0.002 , and $0.061 \pm 0.004 \text{ d}^{-1}$ for phases 1, 2 and 3, respectively. No specific growth rate reduction was therefore evidenced while increasing the percentage of centrate in the feed.

As for the trend in biodiversity in the four reactors, Figure 4 shows the qualitative abundance of the main

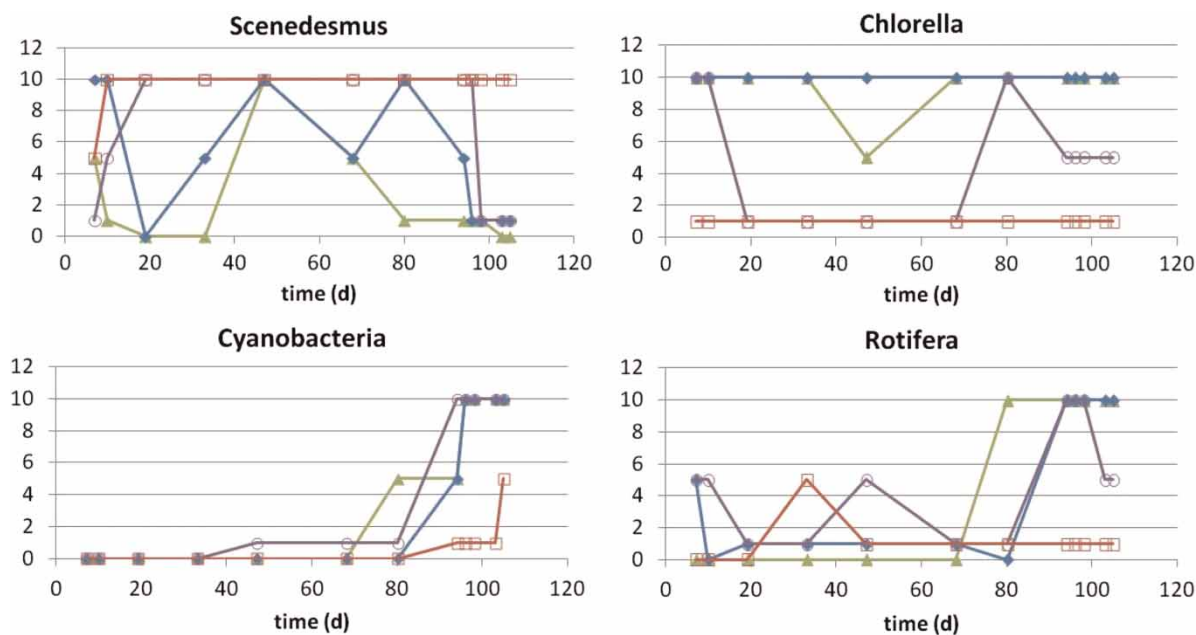


Figure 4 | Abundance (0 = absent; 1 = scarce; 5 = relevant; 10 = abundant) of the main microflora components in the reactors (R1: triangles; R2: diamonds; R3: circles) and column (squares) during the course of the experiment.

organisms. Although initially *Botryococcus* cells were also present in the algal mixture, they were outcompeted by *Chlorella* and *Scenedesmus*. It can be seen that *Scenedesmus* was the prevailing microalgal component in the column and in R3, while a more balanced mixture of both microalgae was observed in R1 and R2. These differences did not seem to show any correlation with either the N-removal efficiency or the SS/N ratio.

During phase 3, an increased presence of undesired cyanobacteria and rotifera was observed in all PVC reactors (R1–4), and, to a lesser extent, in the column.

The lower η_N observed in phase 3 could be therefore due to improved predation by rotifera or to the competition with cyanobacteria for common substrates such as CO_2 or phosphorus. Moreover, other factors, such as light penetration or CO_2 availability, could have played a limiting role during phase 3, with N being the limiting factor in phases 1 and 2.

BMP and settleability tests

Specific tests were performed to assess the algal methane potential and its settleability as such or after blending with waste activated sludge.

Methane production tests were conducted on two algal samples: A1 obtained by mixing samples collected from R1 and R2, and A2 collected from the Plexiglas column. Both samples were previously thickened on a laboratory

centrifuge. The BMP values, referred to normal conditions of temperature and pressure (0°C , 1 atm), were $335 \pm 39 \text{ mL CH}_4/\text{g VS}$, and $284 \pm 68 \text{ mL CH}_4/\text{g VS}$ for A1 and A2, respectively.

As for the settleability tests, results are summarized in Table 4. It can be observed that although the settling capacity of microalgae is satisfactory, nevertheless it can be effectively improved after mixing with activated sludge. These results confirm the potential to use the existing primary settler, into which excess secondary sludge is recirculated, for microalgae thickening.

DISCUSSION

Results from this experimental campaign suggest that no severe inhibition of algal growth was observed by feeding undiluted centrate to an algal suspension made of a mixture of *Scenedesmus* and *Chlorella* for more than three times the

Table 4 | Results of the settling test (A = algal suspension; AS = activated sludge)

	Algal suspension (100% A)	Mixture (30%A + 70%AS)	Mixture (70%A + 30%AS)	Activated sludge (100%AS)
SVI (mL/gSS)	88.6	88.9	86.7	97.8
% of unsettled SS	17.2%	9.5%	8.7%	7.9%

HRT value. The nitrogen removal efficiency decreased slightly for increasing percentage of centrate in the feed, but remained between 77 and 82%. Such values are comparable to literature results. Wang *et al.* (2010), for instance, found 82.8% nitrogen removal efficiency working with *Chlorella* sp. on centrate with 131.5 ± 2.1 mg/L concentration of total nitrogen. Also, the SS production to N removal ratio ($\Delta\text{SS}/\Delta\text{N}$) decreased from 9–11 to 6–7 gSS/gN, which was likely due to improved predation by rotifers. Specific growth rates of $0.04\text{--}0.06\text{ d}^{-1}$ were observed in all reactors and no clear reduction was observed when treating the undiluted centrate. Chinnasamy *et al.* (2009) obtained higher growth rates ($0.065\text{--}0.22\text{ d}^{-1}$) for *Chlorella vulgaris* but worked at higher CO_2 concentrations and at higher temperatures ($30\text{--}50^\circ\text{C}$) and found that the optimal conditions for algal growth were at 30°C and 6% CO_2 concentration. Devgoswami *et al.* (2011) obtained the maximum growth rates of *Chlorella* and *Scenedesmus* (0.704 and 0.673 d^{-1} respectively) with a CO_2 concentration of 4.758 mg/L . In such conditions, the lipid content of all the strains also increased reaching 18% and 14% of dry cell weight, respectively.

The great potential of centrate as a substrate for microalgal growth is confirmed by Wang *et al.* (2010) who compared the results obtained on treated effluents (rich in nitrate nitrogen) and centrate and concluded that centrate, besides providing greater nitrogen availability (as ammonium), also provided the needed phosphorus amount, in spite of the unbalanced N/P ratio. The unbalanced N/P ratio of the centrate was found to affect neither nitrogen nor phosphorus removal, suggesting the importance of the absolute abundance of both nutrients for algal growth, irrespective of the optimal relative ratio.

Moreover, the microalgal suspension proved to have a satisfactory settleability and microalgae were found to have an interesting methane production potential. The obtained BMP data (between 284 and 335 mL $\text{CH}_4/\text{g VS}$) are in agreement with previously reported values (Mussnug *et al.* 2010).

CONCLUSIONS

The experimental results show that the centrate does not induce any toxicity and, on the contrary, can be well utilized by microalgae, whose average specific growth rate (μ), on centrate as such was between 0.04 and 0.06 d^{-1} . The maximum biomass concentration in the photobioreactor effluent was 1.6 gSS/L at 10 days HRT and the nitrogen removal efficiency remained as high as 77–82% when

treating undiluted centrate. These results confirm that algal cultivation on reclaimed, nutrient-rich streams such as those available in wastewater treatment plants is a feasible option. Experimental data also suggest that the existing primary settler could be used for microalgae settling and that the potential of microalgae for methane production in the existing anaerobic digester is also interesting.

Therefore, the proposed integration (see Figure 1) appears feasible, both considering the centrate as substrate and using the CHP off-gas as a CO_2 source, also taking into account the positive effect of high CO_2 supply on algal growth and lipid accumulation in algal cells.

However, further aspects have to be taken into account to finally prove the technical feasibility of this process and identify limitations and constraints on the integration of the photobioreactor into the WWTP scheme. The photobioreactor converts soluble nitrogen into particulate organics which are recycled to the anaerobic digester. In the digester, ammonia is partly released by hydrolysis while a fraction of the recycled N is removed with biosolids. The higher the amount of N recycled from the photobioreactor to the anaerobic digester, the higher the amount of N that will be removed with biosolids, thus reducing the N load to be treated in the water line. By increasing the N recycling, the amount of particulate organics that are loaded to the anaerobic digester will also increase, thus improving the plant energy balance. Nonetheless, the ammonia concentration will also increase to a limit value that will serve as the overall system constraint. The other most relevant constraints will come from surface availability to collect sufficient solar energy, the seasonality of its availability, and the primary settling efficiency. All those aspects, along with a careful COD/energy mass balance, are under study.

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