S1. Supplemental

Hydrology in soil columns

S1.1 System description

The system set up was made of two columns (Figure 1) filled with fine grade soil, with soil average grain size of 0.33mm (US Silica product data). Flow was free for the first 100 min, until saturation rates showed stabilization. Each column length was 1.2m with a diameter of 0.102m. Eight sampling faucets were designed through the column depth at 10cm, 20cm, 30cm, 40cm, 50cm, 70cm, 90cm, and 110cm and at the influent side and output effluent side as detailed at Figure 1. The system set up was made of two columns (see figure 1) filed with fine grade soil; soil average grain size is 0.33mm (US Silica product data). Previous calibrations showed saturation level of the void volume of 75% and 73% at column CC and PC, respectively (from 3 hours after flood start, Figure S2). This study was conducted following previous work (Friedman et al 2017 column 1 paper) when both columns were fed for 5 months with the same feed and showed similar removal profiles before addition of H_2O_2 to PC.

S2.

S2.1 System operating conditions

In each column SE solute was added to the influent in a flow rate of 0.6L /hour for 12 continuous hours. It was controlled by adjusting the outlet flow. To simulate continues flooding conditions, a head was constructed at the top 23 cm above soil level, and was fed every 1.5 hours to avoid any reduction of the solute level under 10 cm. After 12 continues hours of SSE solute flooding the columns aeration period was 36 hours, similar to the operational conditions of SAT treatment in SHAFDAN WWTP. The set-up was also designed to simulate preferable saturated flow path through the unsaturated (vadose) zone, typical to the nature of flow in unsaturated zones beneath artificial recharge basins.

(1) Calculated column area will be $A = \pi \cdot (d/2)^2 = 0.0082\ [m^2]$

(2) Using Darcy's Law, when the flow is constant and controlled, the hydraulic conductivity was calculated: $K = \frac{Q}{A \cdot \frac{\partial h}{\partial x}} = \frac{0.6/1000}{0.0082 \cdot \frac{0.23-0}{1.2}} = 0.383$[m/hr] $=1.06 \cdot 10^{-4}$[m/s]

Q = flow rate $\mapsto$ 600 ml/hr

A = calculated area (πr^2) $\mapsto$ $\pi \times (5.1\ cm)^2 = 81.7\ cm^2$

h = head, flooding condition head above soil level $\mapsto$ 0.23 cm

The hydraulic conductivity resembles the capability of soil to transfer water.[63]

The flow was controlled to enable sufficient residence time for bacterial activity on one hand but also simulate infiltration.

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S3.

S3.1 Dissolved oxygen measurements

Dissolved oxygen was measured using YSI DO probe. The measure at the top was taken by dipping the probe in the solute at the column head and at the bottom was taken in the same sealed continuous flow chamber that was used for the retention time measurements.

S4.

S4.1 Saturation rate over time

The columns saturation rate was calculated by the percentage of the delta volume of the measured inlet and outlet as a fraction of the void volume inside the column. Experiment was done on column CC and PC (control and peroxide column, respectively).
Weight of solution volume was documented continuously at the outlet. At the head, weight was documented every feel (each feel was to the max volume) and throughout the run by measuring the water drop from maximum. The inlet volume (ml) was measured by the reduction of the height (cm) of solution level and multiplied by column area of 81.7cm2 (Figure S3). The outlet volume was measured by weight (g) inside a bucket on a balance.

Figure S1. Experiment Setup to measure saturation rate. The outlet is drained to a bucket which is continuously weight on a balance
The total column volume is calculated by the height (120cm) x the area (81.7cm) =

9805mL. The void volume is calculated by total column volume (9805mL) x pour volume (porosity) calculated by tracer experiments previously (0.33) = 3234 mL
The saturation rate was calculated by formula (1):
Saturation Rate (%) = 100-((Air volume-Average Delta)/ Air volume) *100)

$$\text{Control column} = 1-(\frac{2421}{3234}) = 74.9\% \sim 75\%$$

$$\text{Peroxide treatment column} = 1-(\frac{2345}{3234}) = 72.5\% \sim 73\%$$

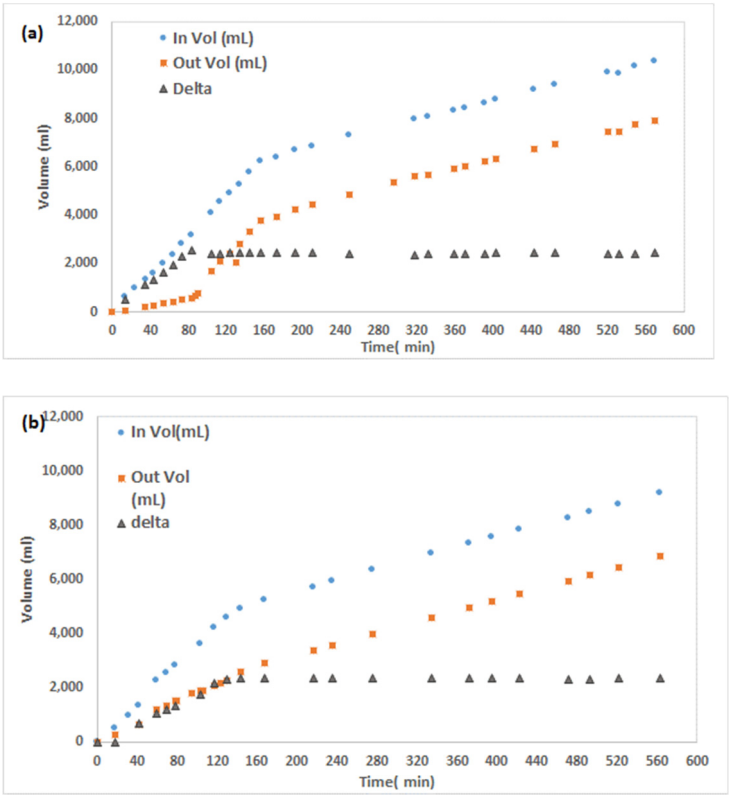


Figure S2. Volume (mL) of inlet, outlet and delta (ml) of Column CC (a) and PC (b) (peroxide treatment) over time (min).

Conclusions

The saturation rate experiments of control and peroxide columns show that delta, the water saturation in the void, was similar and typical for such a SAT system (70- 80%) in both columns and stabilized after about 2 hours of gravitational flow. The column saturation was constant throughout the experiment time. Based on these results the set up can be assumed as steady state from 2 hours of operation and on. Also high availability of oxygen throughout the column can be assumed, as typical for SAT, which also matches dissolved oxygen measurements that was conducted [9,51].

S5.

S5.1 Secondary effluent ions and anions content

The synthetic secondary effluent solution was designed based on Shafdan WWTP secondary effluent annual average values which is characterized with high NH4+ and COD concentrations. The feed content was also similar in values of conductivity, pH, alkalinity, trace elements, ions, cations and phosphorous concentrations (Table S1).

Table S1. Specification of secondary effluent anions and ions

Compound	Concentration	Unit
SO4	26.10	mg/L
PO4	1.33	mg/L as P
HCO3	142.5	mg/L as CaCO3
Cl	331.0	mg/l
Fe	3.30	mg/l
Zn	0.74	mg/l
Mo	0.40	mg/l
Ni	0.14	mg/l
Mn	1.18	mg/l
Cu	0.23	mg/l
Mg	25.12	mg/l
Ca	78.11	mg/l
K	22.45	mg/l
Na	134.0	mg/l
Conductivity	1180±10	μs/cm2

For all measurements, standard and calibration curves were used. Detection limits were determined as ±1 mg/l for COD and ±0.1, ±0.047, ±0.009 mg/l-N for ammonia, nitrite and nitrate respectively.

S5.2 Organic nitrogen content measurement (LB)

A solution of 91 mg LB in 150 ml DI water was diluted at a ratio of 1:100 (1.5 ml/150 ml) to obtain a concentration of 10 mg/L-N. Two samples were measured in duplicates to determine the content of organic N at the diluted solution. The measured values were 6.1 ± 0.31 mg/L N. The calculated value of the N content of LB was 6.7% (6.1 mg-N/91 mg LB). Thus, the N value of organic N at the SSE was 4.62 mg-N/L (69 mg LB × 0.067) and a C/N=5.

Table S2. Concentration, biodegradability and nitrogen content (by weight) of COD and ammonia concentration at the feed. * Measured nitrogen content of LB (Table S4).

COD biodegradability content (mg/L)			COD (mg/L)	N content of COD (mg/L-N)*	NH_4^+ (mg/L-N)	NO_2^- n(mg/L-N)	NO_3^- (mg/L-N)
Humic acid (Slow)	LB broth (Moderate)	Glucose (Fast)					
4.90	37.50	19.60	63.2±1.1	4.5±0.1	4.1±0.2	1.8±0.05	0.22±0.01

S5.3 H2O2-degradation examination in mixed-batch reactors

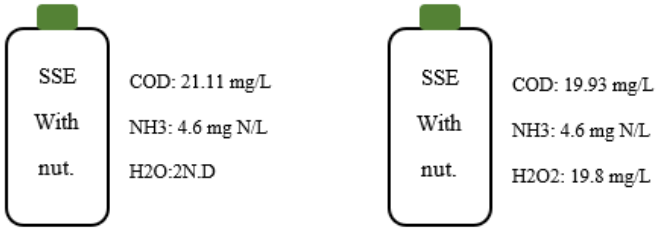
The goal of this experiment was to examine the degradation rate of peroxide and removal of ammonia and COD in inactivated biomass (autoclaved) of soil.

Abbreviation: SE – Synthetic secondary effluent, COD – Chemical oxygen demand (mg/l).

Set up:

Initially, bottles of 1L filled with SSE were prepared following the same procedure of the SSE for feeding columns.

One bottle of SSE with nutrients was also added with peroxide to reach a concentration of 20mg/L.



Scheme S1. The test bottles and content.

Soil samples from the top layer of Column PC (fed SSE and H2O2 for 5 months) was autoclaved and distributed in tea bags. The tea bags were soaked for 1.5 min in their expected solution (SSE).

Six Erlenmeyer of 150 ml were fully covered with aluminum foil to avoid any light interference and were filled with the soil samples and the designed solution. After verifying there is no air bobbles the chambers were sealed with rubber clogs and parafilm foil and held for 7 hours to simulate the residence time at the column. Samples of the solution of each vial were taken for measurements when the experiment was over.

The Erlenmeyers content set up was:

1. SSE without H2O2.

2. SSE with H2O2 and 10 gr of autoclaved soil.

3. SSE with H2O2.

4. SSE with 10 gr of autoclaved soil only.

Dissolved oxygen (probe calibrated purging oxygen in water), NH4+, NO2-, NO3-, COD, H2O2, pH and ions were measured before and after the experiment. All measurements were conducted in duplicate.

Table S3. Concentrations of ammonia, COD, H_2O_2, at start and 7 hours in the sealed vials. Number of the treatment is referred in brackets.

Sample	NH3 (mg/L as N)		COD (mg/L)		H2O2 (mg/L)	
	Conc.	Std. Dev.	Conc.	Std. Dev.	Conc.	Std. Dev.
SSE only (0 Hrs)	4.595	0.043	21.112	0.062	0.026	0.022
(1) SSE only (7 Hrs)	4.521	0.003	20.707	0.927	0.046	0.004
(2) SSE only + A.C. Soil (7 Hrs)	4.359	0.006	20.523	0.154	0.068	0.007
Before SSE+ H_2O_2	4.558	0.046	19.930	0.525	19.768	0.012
(3) SSE+ H_2O_2 (7 Hrs)	4.458	0.020	20.957	0.451	19.036	0.008
(4) SSE+ H_2O_2 + A.C. Soil (7 Hrs)	4.292	0.063	20.320	0.433	17.603	0.006

No significant change in ammonia or COD concentrations was observed in all experiments without soil (Table S3).

In the presence of soil, however, ammonia concentrations showed a slight decreased of 3-4% that can be explained by absorption due to significantly higher surface area by the soil addition. As for soil and peroxide – the was some reduction (~2mg/L) but the reduction rate observed was two orders of magnitude slower than observed in the column set up with vital biomass. This show that soil presence had some influence on peroxide degradation, presumably by absorption or chemical degradation with some carbon dissolved from the autoclaved soil.

No significant change observed in all other parameters measured (pH and DO).

Conclusions:

No significant change in parameters was observed in any of the control in time scale of 7 hours due to the soil presence or the in the mixture of SSE and peroxide.

S6.

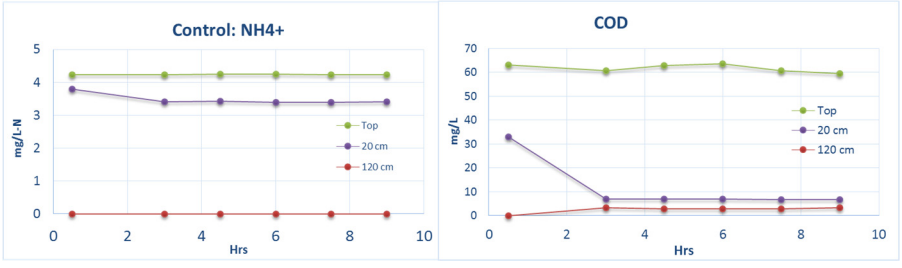
S6.1 Parameters consistency – steady state conditions at measuring campaign

To conclude on a preferable time frame for solute sampling, samples were taken from the column's depths of top (0), -20 cm, bottom (-120 cm). Ammonia and COD was measured as an indication for the microbial activity after 0.5, 3. 4.5, 6, 7.5 and 9 hours.

After 0.5 hour seems like the system was not on full function, which achieved only after 3 hours and on. Sampling time was determined at the middle of the flood cycle – after 6 hours from start.

Peroxide column obtained significantly higher removal rates.

(a)



(b)

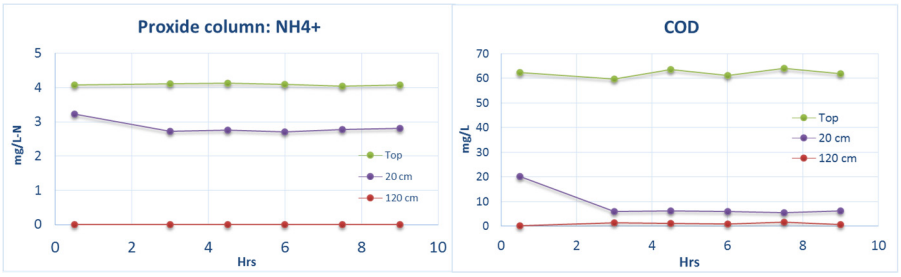
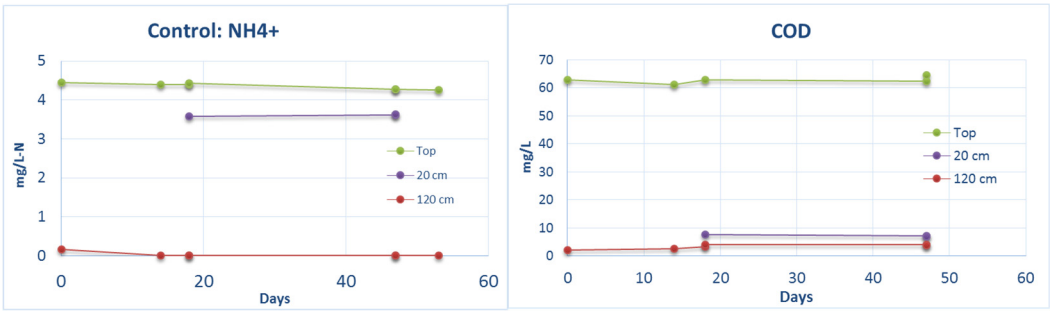


Figure S3. Ammonia and COD values measured over 9 hours at the control (a) and peroxide (b) column.

In order to assume a steady state condition for the system over days, as a plug flow reactor, measurements conducted to verify a constant activity and removal rates. Samples were taken between 6-7 hours after the beginning of the flood cycle from the columns depths of the top, -20cm and bottom (-120 cm) of ammonia and COD over a period of 53 days. All measurements showed steady state over days (Figure S4).

(a)



(b)

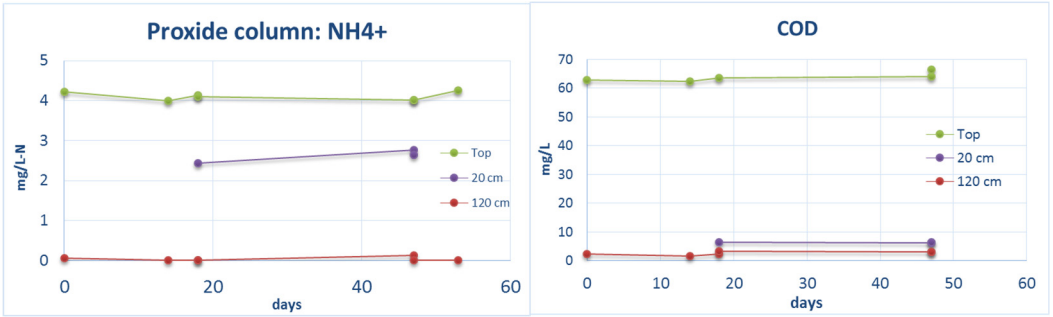


Figure S4. Ammonia and COD values measured from control (a) and peroxide (b) column at the top, -20 cm and – 120 cm.

Although different removal rates were obtained in each column, no significant change was observed over 53 days of operation. The full profile measurements campaign was conducted between 6-7 hours after the beginning of the flood cycle and is consider as steady state (Figure S4).

S7.

S7.1 Molecular methods for microbial community analysis

16S qPCR gene analysis

The functional groups analysis was conducted as described at Park et al., 2015. Standard curves for qPCR analysis was conducted in serial decimal dilution of plasmid DNA containing specific target gene inserts. The qPCR for DNA samples were conducted in triplication (technical significance) for every duplicate of each sample (biological significance). The functional groups analyzed specified herein: AOB by amoA, Nitrospira spp. by NSPRA 16S, Nitrobacter spp. by Nitro 16S, Universal bacteria Eub 16S gene as total bacteria and AMX. AmoA values were normalized considering the template usually appear in two copies.

S7.2 *Microbial community analysis*

In brief, the extracted DNA of every sample was analyzed by next-Generation Sequencing of Amplicon Library of Ribosomal Database Project (RDP), Classifier software was used to classify the 16S gene sequences and assign them to the closest known genus in the NCBI database with an 95% threshold. In addition, after excluding all genus with abundance lower than 1%, every genus was classified under metabolic ability in terms of aerobic and limited oxygen potential activity.

In order to focus on the dominant genus in the samples, all genus with abundance lower than 2% were excluded. The dominant genus which are presented in Fig S10 represent only ~40% of the total community.

S7.3 Bacterial assimilation calculation

To evaluate nitrogen utilization in favor of assimilation, calculations were made considering chemical oxygen demand (COD) values measured and assuming most of the assimilation occurred by heterotrophs using Eq. S2:

Eq. S2. % N assimilation via nitrification=$\%N_{COD} \times Y \times \Delta COD$ _

$\%N_{COD}$ = calculated average content of organic nitrogen from COD =6.9% (Table S5); Y = 0.5. estimated Yield of heterotrophs (OHO) ΔCOD [65], ΔNH4+, ΔNO2- = measured values mg/L-N. The estimated N assimilation rate at CC was 34% and 28% for PC. Calculations of N assimilation are presented in Table S4.

Table S4. Calculations of N assimilation. *Calculated based on total nitrogen measurements (data not shown)

	ΔCOD (mg/L)	**N content of COD (mg/L-N)***	**N loss mg/L**	**N assimilation (mg/L-N)**	N assimilation (mg/L-N) %
CC	59.6	4.4	6.2	2.12	34.0%
HPC	62.6	4.4	7.7	2.15	28.0%

Table S5. Comparison between RC and HPC for the removal of COD, NH_4^+, and N.

	Reference	HPC	Improvement by H_2O_2
COD removal top 10 cm (mg/l)	40.2	48.8	21.5%
NH_4^+ removal top 10 cm (mg/l-N)	1.2	1.6	10.1%
N removal whole column (mg/l-N)	5.0	7.0	20.5%