

EFFICIENCY LIMITING FACTORS OF PETROCHEMICAL WASTEWATER TREATMENT USING HYBRID BIOLOGICAL REACTOR

Mohd Elmuntasir AHMED*, Andrzej MYDLARCZYK, Adel AL-HADDAD

^{1, 2, 3}Water Research Center, Kuwait Institute for Scientific Research, P.O. Box 24885, 13109 Safat, Kuwait

Received 03 November 2021; accepted 28 March 2022

Highlights

- ▶ An up-flow biological reactor filled with bio-carrier, was operated at two flow rates, two dissolved oxygen (DO) levels, and under anaerobic conditions.
- ▶ The highest organic removal efficiencies during the aerobic operation were achieved at OLRs of 0.2 kg-COD/m³/d and HRT of 26.67 h.
- ▶ Under anaerobic conditions, the highest efficiency achieved was 41.7 for both COD and BOD at 0.18 kg-COD/m³/d for the high flow scenario.
- ▶ Under anaerobic conditions, the TOC removal was more stable, although at lower efficiencies, the anaerobic scenario was inferior to the aerobic scenario with respect to COD removal. However, the TOC removal stability could be attributed to removal mechanisms other than biodegradation, as it tapered at high loading.
- ▶ The nutrient removal efficiency was marginal, conceivably due to high organic concentrations, toxic conditions of the wastewater, and high organics to nutrient ratio promoting nutrient removal inside the biofilm.

Abstract. The wastewater characteristics and some operational control parameters limit the efficiency of attached growth processes for petrochemical wastewater treatment. This study aims to determine the efficiency of a hybrid biological reactor treating actual petrochemical wastewater and to identify the efficiency determining factors. An up-flow biological reactor filled with bio-carrier was operated at two flow rates, two dissolved oxygen (DO) levels, and under anaerobic conditions. Due to the varying characteristics of actual petrochemical wastewater, efficiency limitations were manifested in many ways. However, the highest chemical oxygen demand and biochemical oxygen demand (BOD) removal efficiencies were 77.2% and 78.5%, respectively, and were achieved under aerobic operation at organic loading rates (OLRs) of 0.2 kg-COD/m³/d and hydraulic retention time (HRT) of 26.67 h (DO 4.0 mg/l). Anaerobically, the highest efficiency was 41.7 for both at 0.18 kg-COD/m³/d and 400 ml/min. The total organic carbon (TOC) removal stability was attributed to the presence of toxic chemicals and removal mechanisms other than biodegradation, as it tapered off at high loading. The nutrient removal efficiency was marginal, conceivably due to the high organics to nutrient ratio and toxic conditions of the wastewater promoting nutrient removal inside the biofilm.

Keywords: biological treatment, industrial wastewater treatment, integrated film activated sludge, hybrid biological reactors, mixed growth biological processes, petrochemical wastewater, pilot study.

Introduction

Often the efficiency of biological processes for organics removal from refinery and petrochemical wastewater is less than that for the municipal wastewater since the initial organics concentration is higher in the case of petrochemical wastewater (Zaffaroni et al., 2016) and the elevated levels of hard to degrade and toxic organics (Di Fabio et al., 2011). However, the organic pollution load could

be removed effectively using biological processes under the right operating conditions, which are explored in this paper.

In comparison with biological treatment processes, the conventional physical, chemical, and combined treatment methods are associated with high capital and operational cost with respect to their efficiency. For example, Babaei and Ghanbari (2016) investigated the use of ultraviolet (UV)/persulfate and UV/percarbonate, while complete

*Corresponding author. E-mail: miahmed@kISR.edu.kw

decolourization was achieved and enhanced biodegradability was attained, the COD removal efficiency was not more than 70%. Physicochemical processes are also associated with the production of toxic chemicals (Jasper et al., 2017). Hence, further research is needed to develop more environmentally friendly biological processes and approaches to replace energy-demanding physicochemical and conventional treatment techniques (Wollmann et al., 2019). In particular, there is the need to understand the limiting conditions of organics removal from industrial wastewater using biological processes and to identify the optimum operating parameters. Thus, an insight into these systems' operational limitations would be useful for designing, operating, and controlling wastewater biological processes.

Petrochemical wastewater treatment using biological processes is faced with several challenges, affecting the viability of the treatment process to include ease of operation, flexibility to variable loads, the potential for effluent reuse, high levels of toxic and hard to degrade contaminants, and adaptability of microorganisms (Zaffaroni et al., 2016; Cattaneo et al., 2011). Out of these challenges, high concentrations of toxic and hard to degrade contaminants in petrochemical wastewater may have an adverse impact on biomass, which is the backbone of any biological treatment process. For example, a study by Chavan and Mukherji (2010) investigated the use of rotating biological contactors (RBC) with algal biofilm to treat petroleum hydrocarbon containing wastewater. They found that despite the advantages of their system over conventional heterotrophic systems, hydrocarbon co-contaminants in the wastewater inhibited the degradation of total petroleum hydrocarbons (TPH) (Chavan & Mukherji, 2010). Nonetheless, biological treatment systems obtain high performances in terms of organic matter and nutrients removal; because the microbial communities growing in these systems could adapt to the wastewater conditions (Gonzalez-Martinez et al., 2018).

Of particular interest among biological treatment processes is the attached growth or integrated fixed-film process IFAS (Jafari et al., 2013; Naghipour et al., 2020), which in some cases, could be considered an alternative to conventional activated sludge processes for treating organics and nutrients in wastewaters, and in particular, in industrial and petrochemical wastewater (Jin et al., 2015). These processes are applicable at the industry level and integrated into wastewater treatment plant schemes. They also have the advantages of less volatile suspended solids (VSS) and total dissolved solids (TDS) in their effluents (Zaffaroni et al., 2016).

In attached growth processes, the biofilm is composed of complex heterogeneous microbial consortia, which utilize different nutrients and carbonaceous materials and absorb metals from the wastewater by secreting a wide range of enzymes. Biofilm processes offer low operational costs, less impact on the environment when compared to conventional wastewater treatment approaches, optimal HRT tolerance, quick adaptation to changes in the wastewater

environment, highly active heterogeneous biomass with improved ability to break down high-strength pollutants mixtures resulting in lower sludge production, and easy separation and sequential reuse of attached biomass (Machineni, 2019; Sehar & Naz, 2016). In addition, biofilm processes achieve higher organics removal efficiencies, have reduced footprints, less requirement of polishing and clarification for reuse of treated effluent. Additionally, the treated wastewater could enhance meeting the quality regulations for reuse. Moreover, it is well-known that biofilms provide extremely robust environments to their colonizers against external physicochemical and biological stresses due to the matrix presence of the extracellular polymeric substances (EPS) (Machineni, 2019; Sehar & Naz, 2016). For instance, Machineni (2019) demonstrated that a rotating-disk biofilm reactor was up to 600 times more resilient to heavy metal toxicity than suspended *pseudomonas aeruginosa*.

However, the operational conditions and the actual wastewater used confer a selective advantage of the hybrid biological reactor at specific operating conditions, including pH, temperature, organic loading, C:N:P ratio, oxygen levels, hydraulic residence time, and COD/BOD ratio. Correspondingly, organics removal in hybrid attached growth reactors is closely linked to the efficiency of nitrification and denitrification processes and modality of operation, among others.

Numerous investigations have experimented with biofilms to treat wastewater and recover nutrients, heavy metals, and other pollutants (Andersson et al., 2008; Chavan & Mukherji, 2010; Schneider & Topalova, 2013). Some attempts to look into the potential of fixed-film processes, particularly hybrid biological reactors, have demonstrated promising potential in some instances (Gurjar et al., 2019; Wang et al., 2019; Wang et al., 2017; Fu et al., 2016; Rava & Chirwa, 2016). The studies have reported varied operational scenarios, including wastewater strength, organic loading rates, hydraulic residence time, the concentration of toxic, and hard to degrade organic compounds and organics to nutrient ratios.

Gurjar et al. (2019) used synthetic wastewater, where the COD and BOD concentrations were 400 and 210 mg/l, respectively, maintaining a BOD/COD ratio of approximately 2 in a laboratory-scale submerged aerobic fixed film reactor packed with synthetic media for OLRs ranging from 0.37 to 1.26 kg-COD/m³d, the COD and BOD removal efficiencies varying between 85 and 89% and 86 to 94%, respectively. It is worth noting that the wastewater used in this study is representative of normal strength similar to domestic wastewater, and the hydraulic retention times are relatively high (8 to 25 h) (Gurjar et al., 2019).

In a hybrid anaerobic biofilm reactor, Wang et al. (2017) increased the OLR from 3- to 33- kg-COD/m³.d. The COD removal efficiency varied from 91 to 86% for retention times of 55 to 12 h, respectively. The reactor treated high-strength wastewater (influent COD having an average value of 10 g/l). In another study, Wang et al. (2019)

operated a full-scale hybrid vertical anaerobic/aerobic biofilm reactor for vegetable processing wastewater treatment. The reactor hydraulic retention time ranged from 32 to 10 h, with the anaerobic OLR reaching a maximum of 16 kg-COD/m³·d. On average, the reactor removed 90% of the feed COD at an operational temperature of 25 °C. These two studies demonstrated the high retention time value required to achieve reasonable removal efficiencies.

Fu et al. (2016) tested an up-flow pilot aerated biological filter for treating petrochemical wastewater. The median removal efficiency values of COD and ammonia nitrogen (NH₃-N) showed 29.35 and 57.98%, respectively. During the operation time (0–12 days), the COD loading had a median value of 0.76 kg-COD/m³/d. The COD removal efficiency decreased with increasing depth of the reactor because of the loss of biomass activity, in turn, due to biomass specific oxygen uptake rate. Fu et al. (2016) found that 90% of the removal efficiency was due to 140 cm height.

Rava and Chirwa (2016) investigated the effect of fill ratio and biological carrier properties on the performance of a hybrid fixed-film reactor treating coal gasification wastewater. The coal gasification wastewater was diluted to 33% to reduce the toxicity to biomass. The OLR was 3.5 kg-COD/m³·d, and the hydraulic retention time was approximately 33 h. The highest microbial activity was obtained with a 50% carrier fill. The bioreactor achieved 49% and 78% removal efficiencies for COD and phenols, respectively. The ammonia-nitrogen removal was insignificant, given that nitrification did not take place due to heterotrophic bacteria out-competing autotrophic nitrifying bacteria in the biofilm (Rava & Chirwa, 2016). Rava and Chirwa (2016) found that the hydrodynamics and biofilm characteristics (activity, density, diversity, and structure) affected the hybrid biological reactor performance. They identified the biofilm structure as a crucial parameter to achieve stable bioreactor performance; since the biofilm structure played an essential role in the rate of mass transfer of nutrients and organic substrate to the microbial community within the biofilm (autotrophic nitrifiers) and on the surface of the biofilm (heterotrophs). They also found that a thin biofilm was insufficient to support

autotrophic nitrifiers but supported viable heterotrophic nitrifying bacteria.

Consequently, it appeared that there is more to investigate relative to the efficiency of biofilm reactors for treating different types of wastewater and, from a practical point of view, there is a need for in-depth evaluation of the potential biofilm processes during longer treatment durations, in order to replicate or mimic the specificities of petrochemical industrial wastewater. Furthermore, the process should be scaled up to a larger volume to confirm their efficiency and applicability. It is also important to identify and understand the dynamics of biofilm processes and explore their relative limitations and value-added to the treatment of petrochemical wastewater.

Therefore, this paper's main objective is to investigate the limitations and determine factors for organics and nutrient removal from actual petrochemical wastewater using a pilot hybrid biological reactor. The study used actual petrochemical wastewater while varying the operational parameters such as oxygen conditions, hydraulic retention time, and OLRs. As such, this paper identifies optimal hybrid biological reactor operating parameters under actual petrochemical wastewater treatment and adds knowledge on the limitations of the process.

1. Materials and methods

1.1. Experimental setup

The hybrid biological reactor capacity was estimated based on loading rates provided for plastic packing media by Metcalf and Eddy (2014). The hybrid biological reactor was a rectangular vessel of 80×80×60 cm in length, width, and depth, respectively, with a total volumetric capacity of 384 l. Additional headspace of 20 cm was provided on top of the reactor for sample collection (Figure 1). The reactor was packed with a high surface area biological growth carrier (RVT Germany, model RFK 50 L, 51 kg/m³ density, 148 m²/m³ surface area). Air was supplied from the compressors (France, LH5003) through a perforated pipes system located at the bottom of the reactor to ensure that the dissolved oxygen (DO) concentration was uniformly maintained at the desired levels. An oxygen sensor

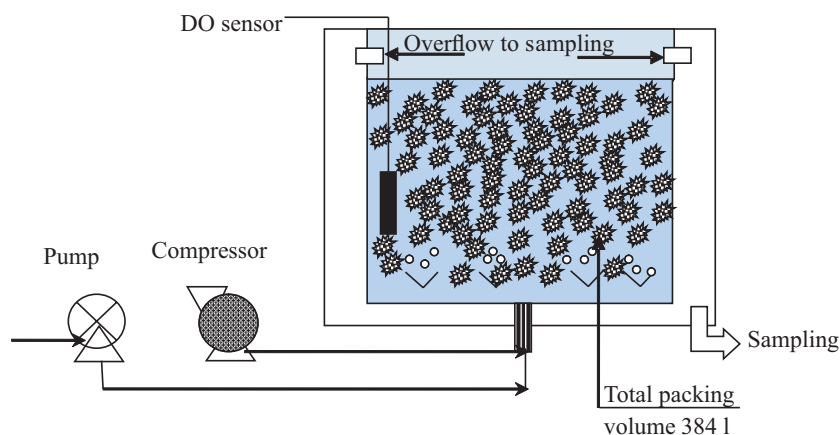


Figure 1. Schematic of reactor setup

was used to monitor the oxygen levels inside the reactor (SONDA DO sensor). Under the anaerobic scenarios, the hybrid reactor was sealed from direct contact with air.

The reactor was fed with petrochemical wastewater from Al-Wafra Industrial Wastewater Treatment Plant in Kuwait using a variable flow pump (MASTERFlex, model 77250-62 L/S™).

The reactor was placed indoors, and the temperature was of room temperature throughout the duration of the experiments. The temperature was also monitored daily throughout the operation period. Aeration and wastewater flow were sufficient to keep the solids suspended inside the reactor.

1.2. Sampling and analysis

Daily wastewater samples were collected and analyzed simultaneously for temperature, pH, DO, BOD, COD, TOC, TSS, nitrogen dioxide (NO₂), nitrate (NO₃), ammonia (NH₃), total nitrogen (TN), total phosphorous (TP), and biomass volatile suspended solid (VSS). The temperature and pH were measured inside the reactor. This analysis was conducted as outlined in the Standard Methods for Water and Wastewater Examination (American Public Health Association, 2014) and the American Standard Testing Methods (American Society for Testing and Materials, 2011).

1.3. Reactor startup

The efficient performance of any fixed-film process is a function of the viability of biofilm development (La Motta, 1976; Bouwers et al., 2000; De Beer & Stoodley, 2006). For commissioning, the reactor was operated for one month, and it was under batch mode for the first 15 days. During this period, it was fed with wastewater acquired from Al-Wafra industrial wastewater treatment plant, and oxygen was maintained above 2.5 mg/l to develop biofilm on the media. In other studies (Mohamad et al., 2017; Kumar et al., 2019), algal strains were used to treat industrial wastewater, and successful removal of NO₃, phosphate, NH₃, and sulfate was achieved within a week of inoculation.

The commissioning was started in batch mode to allow microorganisms to develop on the surface of the media without being washed due to hydraulic shear. The inoculum used for the aerobic scenarios was obtained from the aeration tank, while for the anaerobic scenario, it was obtained from the anaerobic thickeners. To promote initial biofilm growth, quiescent conditions were maintained under batch mode. Once the biofilm was developed, the reactor was operated under continuous mode for the next 15 d. After the priming, the biological film was characterized under a microscope for biofilm coverage and uniformity (Ahmed et al., 2019a).

1.4. Reactor operating mode

The hybrid biological reactor is most commonly used to serve as a replacement for the activated sludge process. In

this study, we are testing its efficiency at different wastewater strengths to understand its optimum operating conditions and efficiency limitations. To meet the study's objectives, wastewater from various petrochemical streams at the inlet ponds of an industrial wastewater treatment plant were used in our experiments to simulate variable loading rates and wastewater characteristics. Since the actual petrochemical wastewater was used, its concentration varies continuously. The wastewater characteristics from various streams, including minimum, maximum, and average, are given in Table 1.

Table 1. Characteristics of the wastewater

Parameter	Units	Max	Min	Average
Temperature	°C	28.8	19.0	22.6±1.9
pH	pH units	8.4	6.3	7.4±0.5
Dissolved Oxygen (DO)	mg/l	8.7	0.2	2.4±1.4
Residual Chlorine (RCl ₂)	mg/l	0.17	0.0	0.0±0.02
Total Suspended Solids (TSS)	mg/l	250.0	2.0	53.4±48.5
Volatile Suspended Solids (VSS)	mg/l	190.0	0.2	40.2±35.8
Total Organic Carbon (TOC)	mg/l	536.1	3.9	97.2±125.8
Chemical Oxygen Demand (COD)	mg/l	1888.4	1.0	532.9±528.6
Biochemical Oxygen Demand (BOD)	mg/l	1059.9	1.5	315.8±307.6
Total Nitrogen (TN)	mg/l	95	8.3	43.5±21.1
Ammonia Nitrogen (NH ₃ -N)	mg/l	68.6	0.0	27.0±20.3
Nitrate Nitrogen (NO ₃ -N)	mg/l	103.0	0.0	6.0±12.4
Nitrite Nitrogen (NO ₂ -N)	mg/l	0.3	0.0	0.03±0.05
Total Phosphorous (TP) (as PO ₄ ³⁻)	mg/l	21.5	0.3	7.0±4.1

After successful commissioning, the reactor was fed with wastewater at various hydraulic loadings and aeration conditions utilizing different wastewater streams. Experiments were typically run for 10 d for each wastewater stream after successful biofilm development.

The results were categorized under six operation scenarios (low flow low air, low flow high air, high flow low air, high flow high air, anaerobic low flow, anaerobic high flow). In each of the six operating scenarios, the experiments were conducted at three different air supply conditions, which were low air (2.0 mg/l DO initial setting), high air (4.0 mg/l DO initial setting), and anaerobic (no air supplied with reactor covered and sealed). The flow was upwards, and for each condition, two flow rates were used; the low flow rate was maintained at 240 ml/s, while the high flow rate was maintained at 400 ml/s in conformity to the operating parameters design given by Metcalf

and Eddy (2014). For each of the six operating scenarios, the samples were collected from the inlet and outlet of the hybrid biological reactor. Samples were collected daily for analysis.

1.5. Biomass characterization

Random packing biological carriers from different zones in the reactor were collected and gently rinsed with distilled water, then dried in the oven at 105 °C for 24 h. The dried carriers were allowed to cool and then were weighed. The attached biomass was removed from the random packing carriers by soaking the carriers in 0.25 N NaOH for 24 h. The carriers were then rinsed very well with water, dried for 24 h at 105 °C, and reweighed. The difference in weight was used to determine the amount of biomass on the carriers. The biofilm thickness was calculated by dividing the total biomass weight by the surface area of the carrier using an approximate dry density of 0.4 g/cm³ (Chang et al., 2005).

2. Results and discussion

2.1. Overview of process performance

The pH during all experiments varied in the range of 6.9 to 7.9 while increasing continuously during each experiment. This range of pH is conformant to the optimum conditions for biological wastewater treatment, according to Baldwin and Campbell (2001). The observed increase in pH indicated no accumulation of acidic intermediates formed during the biodegradation (Chavan & Mukherji, 2010). This pH range could also be suitable for nitrification/denitrification, as well as for microbial activity.

The average VSS content of the random packing material was calculated to be 725 and 327.5 g/m³ for the aerobic and anaerobic conditions, respectively. This indicated that for packing density of 50 kg/m³ and packing surface area of 148 m²/m³, the corresponding biofilm thickness values were calculated to be 12.2 and 5.5 µm, on average, for the aerobic and anaerobic conditions, respectively. These calculated values for biofilm thickness are in the order of 1 µm, as reported by Chang et al. (2005). Therefore, the obtained values in our reactor were satisfactory. Under these conditions, the attached biomass ratio was typically 70% (Ahmed et al., 2019b). The microscopic analysis showed that a good biofilm coverage was obtained and that the biofilm uniformity was also remarkable. This performance was reported earlier by Ahmed et al. (2019c). It is also worth mentioning that no clogging problems were encountered during the reactor operation. The suspended VSS averages were 41.3 and 50.4 mg/l for the aerobic and anaerobic scenarios, respectively. This indicates a higher attached to suspended biomass ratio in the case of the aerobic scenario, which, in turn, supports the advantage of aerobic operation of the hybrid reactor as an attached growth process. It was also noticed that the low flow suspended biomass (average VSS 42.2 mg/l) is slightly higher than the high flow suspended biomass (average VSS

40.3 mg/l), and this indicates a higher washout due to increased inflow.

Table 2 presents an overview of the process efficiency under the six operational scenarios. On average, the COD/BOD ratio was seen to be slightly less than 2, indicating good treatability potential of the petrochemical wastewater. In general, the highest removal efficiency was achieved under the low flow high air scenario and reached 77% removal of COD, similar to results obtained by Gurjar et al. (2019). In contrast, the initial concentration, aeration, and flow rates (hydraulic residence varied greatly as indicated by the ranges and averages of Table 1), similar efficiencies; although of lower values, have been observed for all six scenarios except for the high flow anaerobic scenario with up to 42.3%. It is noticeable that, on average, both scenarios of low flow high air or high flow low air achieved similar COD removal efficiencies (20.9 and 19.9%, respectively), indicative of a strong dependency of the efficiency on OLR and amount of air supplied.

The total nitrogen and total phosphorous varied in the feed concentration up to 95 and 21.5 mg/l, respectively (Table 1). Table 1 also indicates that the average total nitrogen and total phosphorous removal efficiency values were marginal compared to organic removal efficiency. In this case, the petrochemical wastewater may contain relatively small amounts of nitrogen and phosphorous, which may be the limiting step, among other factors, for achieving higher organic and nutrient removal efficiencies (Hamza et al., 2019; Rava & Chirwa, 2016). Vabolienė and Matuzevičius (2005) reported that the ratio of BOD to TP impacts TP removal and that the nitrate in the anaerobic zone has a negative effect on TP removal.

In the different types of experiments (Table 2), the average ratio of COD to TN and TP was more than 100:10:50. This ratio is higher than the optimal ratio of 100: 5: 1 (Metcalf & Eddy, 2014), denoting that nutrients are, in fact, not the limiting factor. Therefore, the insignificant amount of ammonia-nitrogen removed may be due to the fact that nitrification did not take place due to heterotrophic bacteria out-competing autotrophic nitrifying bacteria in the biofilm (due to high nutrient levels), as confirmed by Rava and Chirwa (2016).

Also, it has been reported that the reduction in ammonium-nitrogen was due to evaporative stripping during aeration and not due to nitrification alone (Rava & Chirwa, 2016). This explanation runs parallel with our results, indicating that nitrification/denitrification did not take place, and removal could be due to other physicochemical processes. Additionally, it should be noted that nutrients, as a building block and requirement for microorganisms, are consumed in small amounts, signifying low biological growth and other mechanisms of organics degradation (co-metabolism, etc.). Under these conditions, the biomass in the biofilm could be few in orders greater than the suspended biomass (Bouwers et al., 2000), as was the case in our experiments. Correspondingly, in anaerobic/anoxic/aerobic processes, the anaerobic nitrification/denitrification have taken place ahead of the anoxic phase, and the organic

Table 2. Overview of process efficiency

Parameters	BOD, mg/l		COD, mg/l		TOC, mg/l		TN, mg/l		TP, mg/l		COD Removal Efficiency, %
	In	Out	In	Out	In	Out	In	Out	In	Out	
Low flow low air	3.7–801.0 (319.1)	1.5–675 (307.3)	2.8–1299 (537.1)	1–1076.8 (511.0)	8.6–312 (108.0)	8.3–276 (100.1)	13–70.8 (43.7)	14–71.2 (41.8)	0.8–15.5 (6.8)	0.5–11 (6.1)	1.1–62.3 (13.4)
Low flow high air	9.0–801.0 (318.0)	3.0–675 (286.6)	7.0–1299 (534.0)	2.3–1076.8 (477.9)	8.3–312 (101.2)	8.0–276 (89.0)	19.0–70.8 (47.2)	16.0–71.2 (44.1)	1.5–15.5 (7.2)	0.4–11 (5.7)	1.3–77.2 (20.9)
High flow low air	15.8–880 (395.7)	11.0–756 (302.6)	23–1490 (672.6)	18.0–1293.7 (554.8)	4.7–508.3 (140.7)	3.9–536.1 (145.0)	12.0–66.0 (43.9)	12.9–57 33.6	1.3–21.5 (8.6)	0.5–16.0 (6.0)	1.9–60.0 (19.9)
High flow high air	21.25–1060.0 (440.5)	20.0–986 (383.9)	34.5–1838.2 (745.0)	32.0–1888.4 (690.2)	8.6–499.5 (141.6)	8.7–526.2 (146.6)	13.5–79.8 (45.2)	12.0–67.5 (37.4)	0.3–21.5 (10.1)	1.0–13.2 (7.7)	0.0–67.7 (15.5)
Low flow anaerobic	19.0–656.0 (267.0)	15.0–549.0 (276.8)	31.0–1069.0 (430.9)	25.0–920.0 (449.9)	6.8–239 (58.9)	5.6–196.0 (53.7)	15.0–86.0 (48.4)	13.0–95.0 (50.8)	0.8–11.5 (6.3)	0.3–9.6 (4.9)	0.0–65.8 (14.8)
High flow anaerobic	19.0–656.0 (269.6)	10.0–633.0 (250.4)	31.0–1069.0 (435.9)	16.0–1058.0 (410.9)	8.3–239.0 (56.9)	8.5–101.0 (32.2)	9.4–86.0 (46.8)	8.3–78.0 (43.4)	1.5–11.4 (6.9)	1.0–9.7 (6.4)	0.2–42.3 (12.2)

Note: Table shows range; average (in parenthesis).

substrate in the wastewater has been sequestered by phosphorus-accumulating organisms under anaerobic conditions, resulting in a low or even no availability of organic substrate for denitrifiers under anoxic conditions (Zhang & Gao, 2000). As such, the denitrification performance of the anaerobic/anoxic/aerobic process could sometimes be poor (Zhang & Gao, 2000). Efficient biological nutrient removal requires a sequence of anaerobic-anoxic-aerobic phases with multiple feeding events over one cycle (Puig et al., 2007); and therefore, in our experiments, it would appear that nutrient removal mostly might have taken place inside the biofilm and, to a lesser extent, within the bulk wastewater.

As evident from Table 3, the highest nitrogen removal efficiency of 60.5 and 54.1% occurred during operation under the low flow-anaerobic scenario, and the high flow-high air scenario, respectively, the corresponding COD:N:P ratios were 100:1.7:4.5 and 100:1.6:4.7, respectively. Gonzalez-Tineo et al. (2020), in an aerobic packed-bed with polyethylene rings, attributed nitrogen removal to nitrification (55±11%), denitrification (30%), and to stripping (11±1%). Lee et al. (2002) found that in an aerobic hybrid biological reactor, the efficiencies of total nitrogen (TKN) removal and nitrification were decreased with

increasing COD:N ratio. Therefore, it is conceived that the low flow-high air scenario, since it has the highest OLR, would not perform well in total nitrogen removal. However, since other low flow scenarios had low OLR caused by lower organic concentrations (Table 1), their nitrogen removal was less than would be expected. This is common in operating with actual wastewater, where the concentrations vary continuously.

On the other hand, the highest removal efficiencies for phosphorous were 83.3% for the low flow-high air and 76.9% for the low flow anaerobic scenarios, with the corresponding COD:N:P ratios 100:4.3:7.1 and 100:1.7:6.1, respectively. It was noticed that the highest nutrient removal coincided with the same scenario (low flow anaerobic) for the anaerobic scenario. In contrast, for the aerobic scenario, nitrogen removal favoured the high flow-low air conditions (at COD:N:P ratio of 100:1.6:4.7), and phosphorous removal favoured the low flow-high air conditions (at COD:N:P ratio of 100:4.3:7.1). These observations could be explained in line with the observation of Hamza et al. (2019) in a study using high strength wastewater which showed that the fastest rate of nitrogen removal occurred at COD:N:P ratio of 100:1.1:0.4, which is higher than our hybrid reactor runs (Table 3).

Table 3. Overview of nutrient removal efficiency

Parameter	Operating Mode	Max Removal Efficiency %	Operating Conditions	COD Loading	COD:N:P ratio	COD
Nitrogen	Aerobic	54.1	High flow-high Air	1.93	100:1.6:4.7	1285
	Anaerobic	60.5	Low flow	0.075	100:1.7:4.5	84
Phosphorous	Aerobic	83.3	Low flow-high Air	0.050	100:4.3:7.1	55
	Anaerobic	76.9	Low flow	0.068	100:1.7:6.1	76

A look at the COD average removal of 23.6% for low flow high air, 49.4% high flow high air, 16.7% low flow anaerobic, and 65.8% for the highest anaerobic phosphorous removal could provide a better understanding of the aforementioned observations.

Puig et al. (2007) reported that in a sequencing batch reactor (SBR), biological nutrient removal was successfully achieved by using only one reactor, working with a low organic matter concentration in the influent (C/N/P ratio of 100:12:1.8). All the same, when the C/P ratio was lower than 36 g-COD per g-P-PO₄, an accumulation of phosphate was observed (Puig et al., 2007). Subsequently, the system responded quickly and returned to ideal conditions (C/P ratio of 67 g-COD per g-P-PO₄, taking only 15 d to achieve the complete nutrient removal (Puig et al., 2007). These findings are similar to the hybrid reactor experiments, where the COD/TP ratio was lower than 36; therefore, the nutrient removal efficiency was low.

Table 3 reflects the high phosphorous removal efficiency under the anaerobic conditions (76.9%). While phosphorous removal efficiency in anaerobic processes is low (Sommariva et al., 1997), Keating et al. (2016) have observed successful phosphorous removal (up to 78% of influent phosphate) during the operation of high-rate anaerobic digestion of wastewater; presumably mediated by biofilms in the reactor rather than chemical precipitation. Keating et al. (2016) reported the presence of polyphosphate (polyP) accumulating organisms in his reactor but did not link it to luxury polyP uptake (Solovchenko et al., 2020).

2.2. Effect of operational parameters on process performance

An important factor in the biological treatment was the COD:BOD ratio which, on average, was 1.7 (Table 1). The ratio of these two sum parameters is a traditional measure of the biodegradability of the wastewater (Metcalf & Eddy, 2014). If the COD:BOD ratio does not exceed 2:1, the biodegradability is usually good. Higher values would indicate the presence of hard to degrade organics.

If the wastewater in the inflow to the biological stage is deficient in one of the main nutrients, the organics removal efficiency could be affected (Wang & Wu, 2004). A certain proportion of readily biodegradable carbon compounds (such as BOD) must be present for efficient denitrification. The content of nutrients in the wastewater should cater to the needs of the bacteria in the activated sludge, and a balanced relationship between C, N, and P should be achieved. This is crucial to the effectiveness of the biodegradation processes. During aerobic wastewater treatment, the C:N:P ratio should be in the range between 100:10:1 and 100:5:1 (Metcalf & Eddy, 2014). Moreover, the presence of high amounts of organics could lead to the dominance of the fast-growing heterotrophs (Rava & Chirwa, 2016), leading to poor nutrient removal because the bacterial behaviour in degrading the organics is altered under nutrient-deficient conditions, where faster

degradation rates are observed as the amounts of nutrients decrease, with a higher relative abundance of heterotrophs and diazotrophic bacterial populations (Rava & Chirwa, 2016).

It has been documented that the efficiency of biological treatment processes is dependent on the type and strength of wastewater, concentration of pollutants, OLR, oxygen supply, and nutrient content (Andersson et al., 2008; Chavan & Mukherji, 2010; Schneider & Topalova, 2013; Gurjar et al., 2019; Wang et al., 2019; Wang et al., 2017; Fu et al., 2016; Rava & Chirwa, 2016). The OLR, in turn, is dependent on the wastewater organic concentration and flow and likewise affects the hydraulic residence time, cell residence time, and biomass detachment rates (Metcalf & Eddy, 2014).

Due to enhanced degradation (higher biomass levels) at high OLR, the oxygen demand increases, causing a drop in DO levels (Borghei et al., 2008). Therefore, the DO concentration was monitored daily *insitu*, and the compressor air was adjusted accordingly in all experiments to maintain the DO at the desired levels.

2.3. Aerobic operation scenarios

Figure 2 present the efficiency of the hybrid biological reactor under the low flow low oxygen scenario operating scenario. Figure 2a illustrates the variation of efficiency and VSS with OLR, while nutrient ratio variation is shown in Figure 2b. While the efficiency and nutrient ratio are higher at lower loading rates, the VSS increases at higher loading rates indicating favourable conditions for suspended growth.

Hamza et al. (2019), using SBR, found that the amount of nutrients needed for biomass growth does not follow the conventional organics to nutrients ratio (COD:N:P) of 100:5:1 when dealing with high-strength organics wastewater. As expected, in the low flow low air scenario (Figure 2a), the highest efficiencies were achieved at low OLRs of 0.02 kg-COD/m³/d at around 59.7 and 62.3 for BOD and COD, respectively. These removal efficiencies occurred at the highest nutrient/COD ratios 2.1 and 7.5 for TP and TN, respectively (Figure 2b). At higher OLRs, the removal efficiency dropped significantly, apparently limited by nutrient ratios (0.009 and .05 for TP and TN, respectively). However, the suspended biomass manifested an increase indicative that nutrients are still sufficient to sustain suspended biomass growth even while they could not sustain biofilm growth.

Also, according to Figure 2a, the suspended biomass concentration in the hybrid reactor increased with the increase of OLR. Lee et al. (2002) found that in a hybrid biological reactor, the biomass increase with increasing OLR as well. Noting that the biomass fixed in the carriers predominates in the reactor at all OLR (Wang et al., 2000), the attached biomass played the major role in COD removal.

Ding et al. (2018), in an aerated biological filter treating petrochemical wastewater, found that COD removal

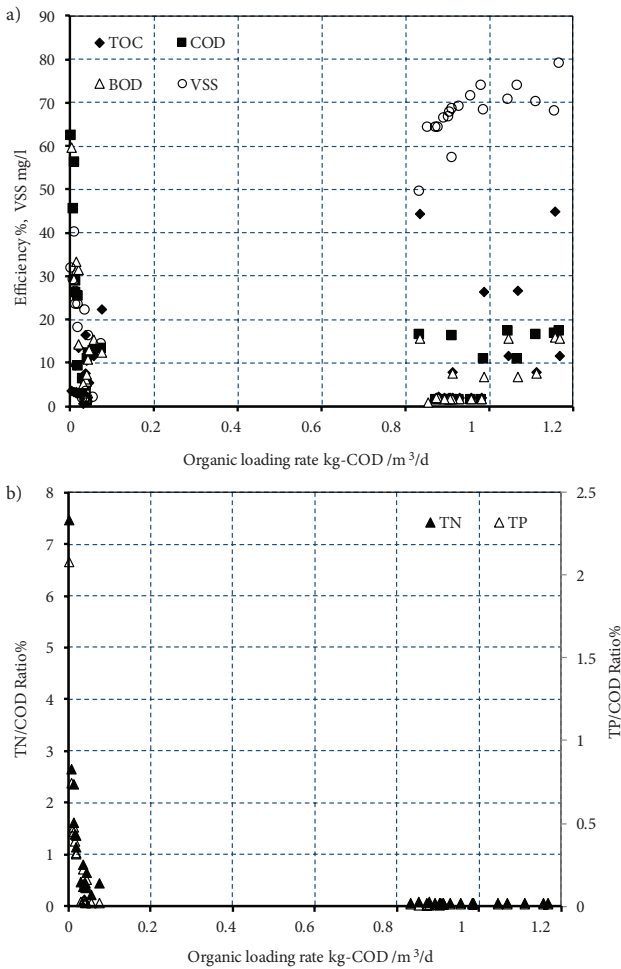


Figure 2. Hybrid biological reactor performance under low flow-low oxygen scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

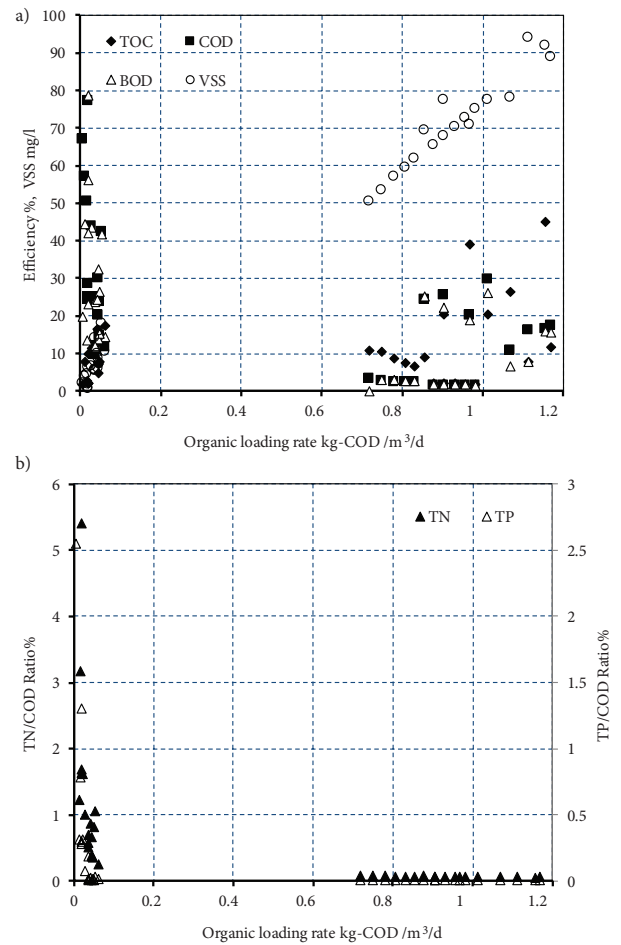


Figure 3. Hybrid biological reactor performance under low flow-high oxygen scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

could be improved by using ozonation coupled with the aerated biological filter since it degrades hard to degrade organics. However, their HRT was 4 h resulting in 27–32% COD removal efficiency, while the HRT in this study was 26.67 h for the low flow case. This illustrates the role of HRT in achieving high efficiencies, as confirmed by Lusnier et al. (2021). In their study (Lusnier et al., 2021) using synthetic wastewater, a COD removal rate above 95% was achieved at hydraulic retention times (HRT) of 24 h and 18 h, and the study found that only the fixed bed hybrid biological reactor was able to maintain high removal efficiency at an HRT of 12 h.

The TOC removal efficiency increased to a highest 45% at 1.15 kg-COD/m³/d and then dropped at higher loading rates. At low loading rates, the degradation of readily degradable organics (e.g. BOD) was generally more efficient, while the hard to degrade organics could have been removed by other mechanisms (Rava & Chirwa, 2016). Apparently, some of the petrochemical compounds which are hard to biodegrade are removed via stripping and other mechanisms (Rava & Chirwa, 2016), and at higher loading, these mechanisms were found insignificant compared

to the total organic load. However, the presence of toxic compounds in petrochemical wastewater is not high since the BOD/COD ratio was 1.7, and according to Babaei and Ghanbari (2016), this wastewater could be treated biologically.

Upon increasing the DO levels to 4.0 mg/l, the organic removal efficiency reached its highest at 0.2 kg-COD/m³/d of 77.2 and 78.5 for COD and BOD, respectively (Figure 3a). Similar to the low flow-low oxygen conditions, the nutrient ratio decreased with increasing OLR (Figure 3b), indicating limitation by COD/nutrient ratio. The increased DO levels have improved the organics removal efficiency, albeit did not change the OLR at which it occurred, indicative that the OLR was the determining factor. The TOC removal behaviour remained the same, with a high 45% at 1.94 kg-COD/m³/d.

In the high flow-low DO case (Figure 4a), the organics removal efficiency was lower (59.5 for both BOD and COD, respectively at 0.2 kg-COD/m³/d) than that of the low flow case. The COD nutrient ratio decreased at higher OLR (Figure 4b), emphasizing the fact that nutrients are limiting at higher organic content or high strength

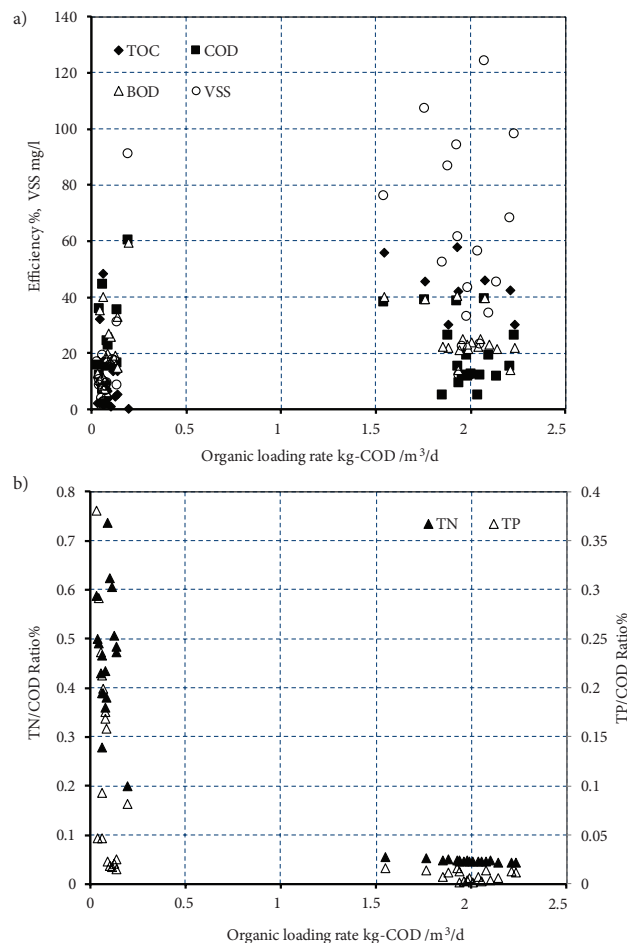


Figure 4. Hybrid biological reactor performance under high flow-low oxygen scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

petrochemical wastewater. This observation was also valid at high DO cases (Figure 5a), where the removal efficiency was 68.3% for both BOD and COD at 0.2 kg-COD/m³/d in comparison to both the low flow low DO and high flow low DO scenarios. This could reflect the disproportionate biomass available due to wash out and the lower nutrient to COD ratios (0.16 and 0.38 for TN and TP, respectively) (Figure 5b) in comparison to the low flow scenarios. The TOC removal efficiency declined at higher OLRs as well. Also, according to Rava and Chirwa (2016), higher oxygen concentrations may lead to reduced carbon removal due to lower nitrification caused by outcompeting the autotrophic nitrifying bacteria in the heterogeneous biofilm by the heterotrophic bacteria and reduced biofilm thickness. Rava and Chirwa (2016) findings explain the higher removal efficiencies at low DO.

2.4. Anaerobic operation scenarios

Anaerobic wastewater treatment has been increasingly used recently, and, in the past few years, a number of experimental studies aimed at improving the performance

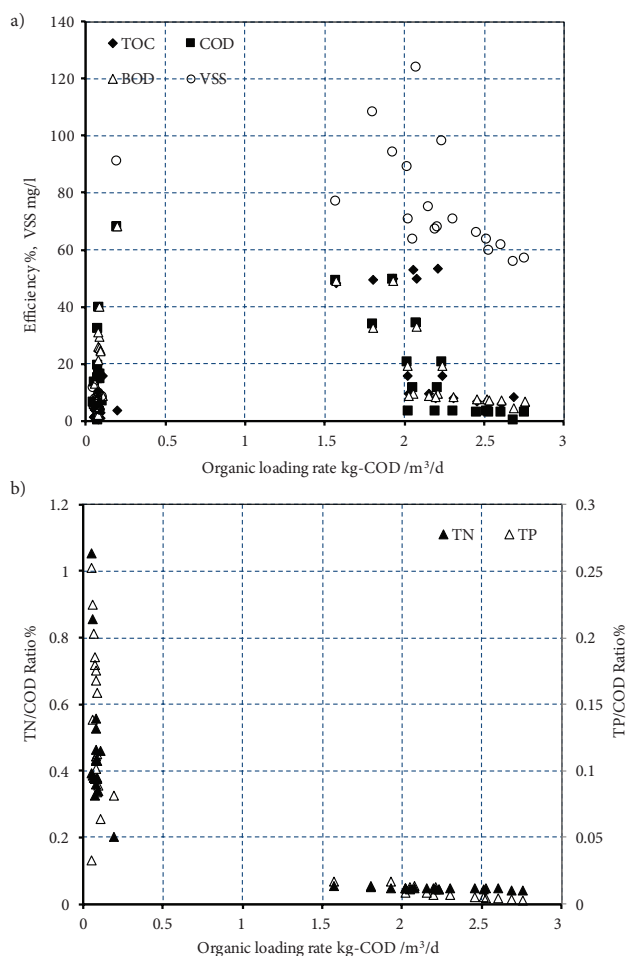


Figure 5. Hybrid biological reactor performance under high flow-high oxygen scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

of the anaerobic treatment process have been reported (Wang et al., 2019; Shoukat et al., 2019; Burman & Sinha, 2020). In anaerobic treatment, the organic matter is transformed by microorganisms into biogas (primarily methane and carbon dioxide) in the absence of oxygen. Some of the reported comparative advantages of this process include lower energy requirement, less sludge generation, lower cost of post-treatment and generation of biogas (Ghangrekar & Behera, 2014; Khalili et al., 2000). However, anaerobic treatment alone cannot always meet the discharge requirements and requires post-treatment using, for example, the anaerobic process (Metcalf & Eddy, 2014; Tomei et al., 2016; Show & Lee, 2017).

For the anaerobic scenarios (Figures 6–7), except for TOC, organics removal rates were lower than the previous scenarios for reasons of lower nutrient content (Figures 6b, 7b), process mechanisms, residence time, and biomass. Similar low efficiencies (35–45% for 4–7 days) at higher OLRs were observed by (Gurjar et al., 2019) and Patel and Madamwar (2002) while operating anaerobic fixed film reactors. The low flow

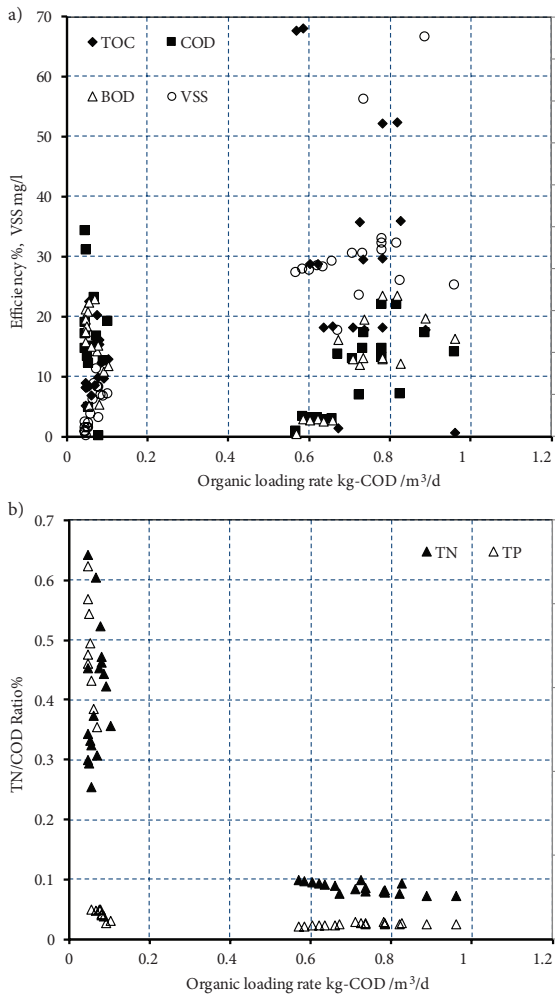


Figure 6. Hybrid biological reactor performance under anaerobic low flow scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

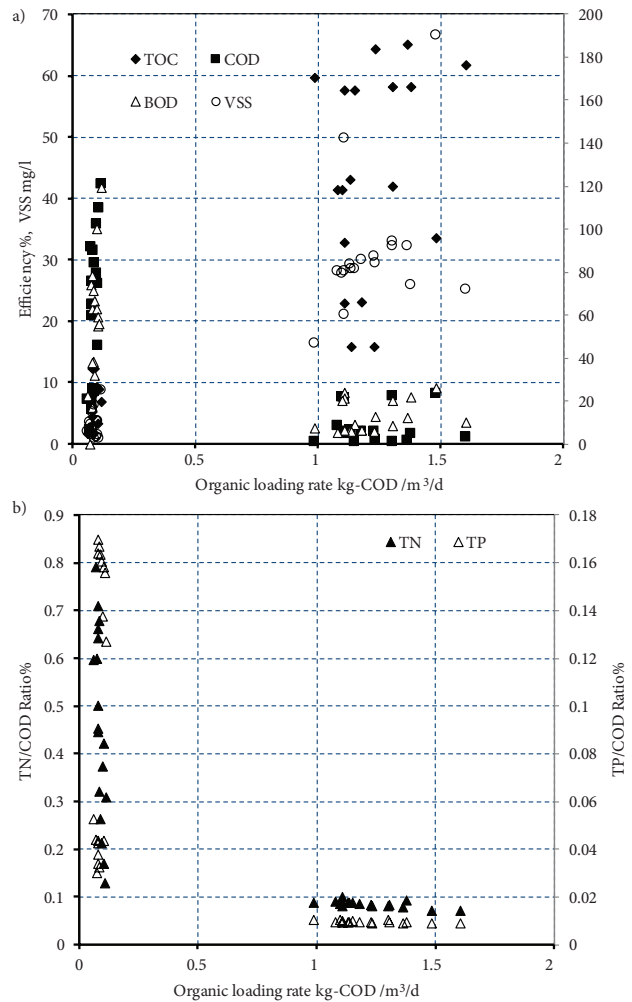


Figure 7. Hybrid biological reactor performance under anaerobic high flow scenario: a) organics removal efficiency and VSS concentration versus organic loading rate; b) TN/COD, TP/COD, versus organic loading rate

case highest organics removal efficiencies achieved were 34.2% and 21.2% for COD and BOD at 0.046 and 0.082 kg-COD/m³/d respectively; while, for the high flow rate, the highest efficiency achieved was 41.7% for both COD and BOD at 0.18 kg/m³/d (Figures 6a, 7a).

TOC removal efficiencies were higher on average and were more stable than COD removal efficiencies. In the low flow operating scenario, 68% was removed at OLR of 0.584 kg/m³/d; while, for the high flow scenario (Figure 6a), 65.1% removal was achieved at 1.365 kg/m³/d. These TOC removal efficiencies may be said to be superior to the aerobic operation, considering that the nitrogen was much higher than the aerobic cases.

In summary, the use of hybrid biological reactors was seen to have achieved its best organics removal efficiency under aerobic conditions and better efficiencies at low flow (low OLR) and high oxygen levels (4.0 mg/l). The flow limited the efficiency as it increased the OLR, decreased the COD/nutrient ratio, and washed out the biomass. In terms of the hybrid reactor

ability to remove hard to degrade organics, obviously, their removal mechanisms are not solely biodegradation; instead, they have complemented stripping and/or evaporation (Rava & Chirwa, 2016).

Under the operating scenarios investigated, seemingly, the best operating OLR was 0.2 kg-COD/m³/d, maintaining nutrient values above 100:5:1 for TN and TP, respectively, under low flow-high DO case. This OLR is within the range cited by Metcalf and Eddy (2014) for plastic media (0.1-0.6). Under these conditions, the process organic removal efficiency could reach 77.2% and 78.5% for COD and BOD, respectively. Notably, the lower efficiencies at higher OLRs in the other scenarios may be feasible as the process could have many advantages, such as lower residence time (smaller reactor volume requirement) and more stable TOC removal. The only benefit of using the anaerobic scenario is the methane production, by which, if coupled with early stages of treatment such as primary treatment, could be of value. Also, the anaerobic operation enhanced TOC removal efficiencies.

Conclusions

The operation of the pilot hybrid biological reactor treating petrochemical wastewater was achieved at different operating conditions for a total of 240 d at room temperatures. The reactor achieved COD removal efficiencies above 77.2% and 41.7% for the aerobic and anaerobic operation scenarios, respectively. Results obtained from this study showed that a hybrid biological reactor would be more efficient in treating petrochemical wastewater at low OLR. The reactor efficiency could be optimized by controlling operational parameters such as the OLR, organics to nutrient ratio, and reduction of toxic and hard to degrade organics. The nutrient removal efficiency was marginal compared to organics removal, conceivably due to the toxic conditions of the wastewater due perhaps to high organic concentrations, toxic conditions of the wastewater, and high organics to nutrient ratio promoting nutrient removal inside the biofilm.

References

- Ahmed, M., Al-Dhafaeri, A., & Mydlarczyk, A. (2019a). Predominance of attached versus suspended growth in a mixed-growth continuous-flow biological reactor treating primary-treated petrochemical wastewater. *Arabian Journal for Science and Engineering*, 44, 4111–4117. <https://doi.org/10.1007/s13369-018-3315-y>
- Ahmed, M., Al-Yaseen, R., Mydlarczyk, A., & Al-Haddad, A. (2019b). Potential use scenarios of hybrid biological reactor for petrochemical industry wastewater treatment. *International Journal of Science and Development*, 10(8), 231–235. <https://doi.org/10.18178/ijesd.2019.10.8.1178>
- Ahmed, M., Mydlarczyk, A., & Abusam, A. (2019c). Laboratory assessment of GAC-Packed IFAS for treatment of primary treated petrochemical wastewater. *Journal of Environmental Biology*, 40, 460–467. [https://doi.org/10.22438/jeb/40/3\(SI\)/Sp-09](https://doi.org/10.22438/jeb/40/3(SI)/Sp-09)
- American Public Health Association. (2014). *Standard methods for examination of water and wastewater*. Washington, D. C., USA.
- American Society for Testing and Materials. (2011). *American standard testing methods*. West Conshohocken, PA, USA.
- Andersson, S., Rajarao, G., Johan Land, C., & Dalhammar, G. (2008). Biofilm formation and interactions of bacterial strains found in wastewater treatment systems. *FEMS Microbiology Letters*, 283(1), 83–90. <https://doi.org/10.1111/j.1574-6968.2008.01149.x>
- Babaei, A. A., & Ghanbari, F. (2016). COD removal from petrochemical wastewater by UV/hydrogen peroxide, UV/per-sulfate and UV/percarbonate: Biodegradability improvement and cost evaluation. *Journal of Water Reuse and Desalination*, 6(4), 484–494. <https://doi.org/10.2166/wrd.2016.188>
- Baldwin, D., & Campbell, C. (2001). Short-term effects of low pH on the microfauna of an activated sludge wastewater treatment system. *Water Quality Research Journal of Canada*, 36, 519–535. <https://doi.org/10.2166/wqrj.2001.028>
- Borghei, S. M., Sharbatmaleki, M., Pourrezaie, P., & Borghei, G. (2008). Kinetics of organic removal in fixed-bed aerobic biological reactor. *Bioresour Technology*, 99, 1118–1124. <https://doi.org/10.1016/j.biortech.2007.02.037>
- Bouwer, E. J., Rijnaarts, H. H. M., & Cunningham, A. B. (2000). Biofilms in porous media. In J. Bryers (Ed.), *Biofilms II: Process analysis and applications* (pp. 123–158). Wiley-Liss Inc.
- Burman, I., & Sinha, A. (2020). Performance evaluation and substrate removal kinetics in an up-flow anaerobic hybrid membrane bioreactor treating simulated high-strength wastewater. *Environmental Technology*, 41(3), 309–321. <https://doi.org/10.1080/09593330.2018.1498132>
- Cattaneo, S., Marciano, F., Masotti, L., Vecchiato, G., Verlicchi, P., & Zaffaroni, C. (2011). Efficacy and reliability of upgraded industrial treatment plant at Porto Marghera, near Venice, Italy, in removing nutrients and dangerous micropollutants from petrochemical wastewaters. *Water Environment Research*, 83(8), 739–749. <https://doi.org/10.2175/106143011X12928814445177>
- Chang, H. T., Parulekar, S. J., & Ahmed, M. (2005). A dual-growth kinetic model for biological wastewater reactors. *Biotechnology Progress*, 21, 423–431. <https://doi.org/10.1021/bp0300671>
- Chavan, A., & Mukherji, S. (2010). Performance of a laboratory-scale RBC with algal-bacterial biofilm treating petroleum hydrocarbon-rich wastewater. *Journal of Chemical Technology and Biotechnology*, 85, 851–859. <https://doi.org/10.1002/jctb.2378>
- De Beer, D., & Stoodley, P. (2006). Microbial biofilms. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes*. Springer. https://doi.org/10.1007/0-387-30741-9_28
- Di Fabio, S., Cavinato, C., Bolzonella, D., Vecchiato, G., & Fatone, F. (2011). Cycling batch vs continuous enrichment of endogenous nitrifiers in membrane bioreactors treating petrochemical wastewater. *Desalination and Water Treatment*, 35(1–3), 131–137. <https://doi.org/10.5004/dwt.2011.3132>
- Fu, L. Y., Wu, C. Y., Zhou, Y. X., Zuo, J. E., & Ding, Y. (2016). Treatment of petrochemical secondary effluent by an up-flow biological aerated filter (BAF). *Water Science & Technology*, 73(8), 2031–2038. <https://doi.org/10.2166/wst.2016.049>
- Ghangrekar, M. M., & Behera, M. (2014). Wastewater treatment and reuse. In S. Ahuja (Ed.), *Comprehensive water quality and purification* (pp. 74–89). Elsevier. <https://doi.org/10.1016/B978-0-12-382182-9.00087-6>
- Gonzalez-Martinez, A., Sihvonen, M., Muñoz-Palazon, B., Rodriguez-Sanchez, A., Mikola, A., & Vahala, R. (2018). Microbial ecology of full-scale wastewater treatment systems in the Polar Arctic Circle: Archaea, Bacteria and Fungi. *Scientific Reports*, 8, 2208. <https://doi.org/10.1038/s41598-018-20633-5>
- Gonzalez-Tineo, P., Durán-Hinojosa, U., Delgado-Mirquez, L., Meza-Escalante, E., Gortáres-Moroyocui, P., Ulloa-Mercado, R., & Serrano-Palacios, D. (2020). Performance improvement of an integrated anaerobic-aerobic hybrid reactor for the treatment of swine wastewater. *Journal of Water Process Engineering*, 34, 101164. <https://doi.org/10.1016/j.jwpe.2020.101164>
- Gurjar, R., Akshay Shende, D., & Pophali, G. R. (2019). Treatment of low strength wastewater using compact submerged aerobic fixed film (SAFF) reactor filled with high specific surface area synthetic media. *Water Science and Technology*, 80(4), 737–746. <https://doi.org/10.2166/wst.2019.316>
- Hamza, R. A., Zaghoul, M. S., Iorhemen, O. T., Sheng, Z., & Tay, J. H. (2019). Optimization of organics to nutrients (COD:N:P) ratio for aerobic granular sludge treating high-strength organic wastewater. *Science of the Total Environment*, 650(2), 3168–3179. <https://doi.org/10.1016/j.scitotenv.2018.10.026>

- Jafari, J., Mesdaghinia, A., Nabizadeh, R., Farrokhi, M., & Mahvi, A. (2013). Investigation of Anaerobic Fluidized Bed Reactor/Aerobic Moving Bed Bio Reactor (AFBR/MMBR) system for treatment of currant wastewater. *Iranian Journal of Public Health*, 42(8), 860–867.
- Jasper, J. T., Yang, Y., & Hoffmann, M. R. (2017). Toxic byproduct formation during electrochemical treatment of latrine wastewater. *Environmental Science and Technology*, 51(12), 7111–7119. <https://doi.org/10.1021/acs.est.7b01002>
- Jin, R., Liu, G., Wang, J., Li, J., & Zhou, J. (2015). Microbial community dynamics in hybrid biological reactor treating petrochemical wastewater. *Desalination and Water Treatment*, 55(5), 1200–1208.
- Keating, C., Chin, J., Hughes, D., Manesiotis, P., Cysneiros, D., Mahony, T., Smith, C., McGrath, J., & O'Flaherty, V. (2016). Biological phosphorus removal during high-rate, low-temperature, anaerobic digestion of wastewater. *Frontiers in Microbiology*, 7, 226. <https://doi.org/10.3389/fmicb.2016.00226>
- Khalili, N. R., Chaib, E., Parulekar, S. J., & Nykiel, D. (2000). Performance enhancement of batch aerobic digesters via addition of digested sludge. *Journal of Hazardous Materials*, 76(1), 91–102. [https://doi.org/10.1016/S0304-3894\(00\)00172-2](https://doi.org/10.1016/S0304-3894(00)00172-2)
- Kumar, P. K., Krishna, S. V., Naidu, S. S., Verma, K., Bhagawan, D., & Himabindu, V. (2019). Biomass production from microalgae *Chlorella* grown in sewage, kitchen wastewater using industrial CO₂ emissions: comparative study. *Carbon Resources Conversion*, 2(2), 126–133. <https://doi.org/10.1016/j.crcon.2019.06.002>
- La Motta, E. J. (1976). Kinetics of continuous growth cultures using the logistic growth curve. *Biotechnology & Bioengineering*, 18, 1029–1032. <https://doi.org/10.1002/bit.260180715>
- Lee, H., Park, S., & Yoon, T. (2002). Wastewater treatment in a hybrid biological reactor using powdered minerals: Effects of organic loading rates on COD removal and nitrification. *Process Biochemistry*, 38(1), 81–88. [https://doi.org/10.1016/S0032-9592\(02\)00044-4](https://doi.org/10.1016/S0032-9592(02)00044-4)
- Lusinier, N., Seyssiecq, I., Sambusiti, C., Jacob, M., Lesage, N., & Roche, N. (2021). A comparative study of conventional activated sludge and fixed bed hybrid biological reactor for oilfield produced water treatment: Influence of hydraulic retention time. *Chemical Engineering Journal*, 420(2), 127611. <https://doi.org/10.1016/j.cej.2020.127611>
- Machineni, L. (2019). Review on biological wastewater treatment and resources recovery: Attached and suspended growth systems. *Water Science & Technology*, 80(11), 2013–2026. <https://doi.org/10.2166/wst.2020.034>
- Metcalf & Eddy. (2014). *Wastewater engineering, treatment, and resource recovery* (3rd ed.). McGraw-Hill Publishers.
- Mohamad, S., Almomani, F., Judd, S., Bhosale, R., Kumar, A., Gosh, U., & Khraisheh, M. (2017). Advanced wastewater treatment using microalgae: Effect of temperature on removal of nutrients and organic carbon. *IOP Conference Series: Earth and Environmental Science*, 67, 012–032. <https://doi.org/10.1088/1755-1315/67/1/012032>
- Naghipour, D., Rouhbakhsh, E., & Jafari, J. (2020). Application of the biological reactor with fixed media (IFAS) for removal of organic matter and nutrients in small communities. *International Journal of Environmental Analytical Chemistry*, 1–11. <https://doi.org/10.1080/03067319.2020.1803851>
- Patel, H., & Madamwar, D. (2002). Effects of temperatures and organic loading rates on biomethanation of acidic petrochemical wastewater using an anaerobic upflow fixed-film reactor. *Bioresource Technology*, 82(1), 65–71. [https://doi.org/10.1016/S0960-8524\(01\)00142-0](https://doi.org/10.1016/S0960-8524(01)00142-0)
- Puig, S., Corominas, L., Balaguer, M. D., & Colprim, J. (2007). Biological nutrient removal by applying SBR technology in small wastewater treatment plants: Carbon source and C/N/P ratio effects. *Water Science Technology*, 55(7), 135–141. <https://doi.org/10.2166/wst.2007.137>
- Rava, E., & Chirwa, E. (2016). Effect of carrier fill ratio on biofilm properties and performance of a hybrid fixed-film bioreactor treating coal gasification wastewater for the removal of COD, phenols and ammonia-nitrogen. *Water Science & Technology*, 73(10), 2461–2467. <https://doi.org/10.2166/wst.2016.108>
- Schneider, I., & Topalova, Y. (2013). Microbial structure and functions of biofilm during wastewater treatment in the dairy industry. *Biotechnology & Biotechnological Equipment*, 27(3), 3782–3786. <https://doi.org/10.5504/BBEQ.2013.0015>
- Sehar, S., & Naz, I. (2016). Role of the biofilms in wastewater treatment. In D. Dhanasekaran (Ed.), *Microbial biofilms – importance and applications*. Intehopen Publishers. <https://doi.org/10.5772/63499>
- Shoukat, R., Khan, S., J., & Jamal, Y. (2019). Hybrid anaerobic-aerobic biological treatment for real textile wastewater. *Journal of Water Process Engineering*, 29, 100804. <https://doi.org/10.1016/j.jwpe.2019.100804>
- Show, K. Y., & Lee, D. J. (2017). Anaerobic treatment versus aerobic treatment. In *Current developments in biotechnology and bioengineering* (pp. 205–230). Elsevier. <https://doi.org/10.1016/B978-0-444-63665-2.00008-4>
- Solovchenko, A., Gorelova, O., Karpova, O., Selyakh, I., Semenova, L., Chivkunova, O., Baulina, O., Vinogradova, E., Pugacheva, T., Scherbakov, P., Vasilieva, S., Lukyanov, A., & Lobakova, E. (2020). Phosphorus feast and famine in cyanobacteria: Is luxury uptake of the nutrient just a consequence of acclimation to its shortage? *Cells*, 9(9), 1933. <https://doi.org/10.3390/cells9091933>
- Sommariva, C., Converti, A., & Del Borghi, M. (1997). Increase in phosphate removal from wastewater by alternating aerobic and anaerobic conditions. *Desalination*, 108(1–3), 255–260. [https://doi.org/10.1016/S0011-9164\(97\)00033-7](https://doi.org/10.1016/S0011-9164(97)00033-7)
- Tomei, M. C., Mosca Angelucci, D., & Levantesi, C. (2016). Two-stage anaerobic and post-aerobic mesophilic digestion of sewage sludge: Analysis of process performance and hygienization potential. *Science of the Total Environment*, 545–546, 453–464. <https://doi.org/10.1016/j.scitotenv.2015.12.053>
- Vabolienė, G., & Matuzevičius, A. (2005). Investigation into biological nutrient removal from wastewater. *Journal of Environmental Engineering and Landscape Management*, 13, 171–181. <https://doi.org/10.3846/16486897.2005.9636868>
- Wang, J., & Wu, L. B. (2004). Wastewater treatment in a hybrid biological reactor (HBR): Nitrification characteristics. *Bio-medical and Environmental Sciences*, 17(3), 373–379.
- Wang, J., Shi, H., & Yi, Q. (2000). Wastewater treatment in a hybrid biological reactor (HBR): Effect of organic loading rates. *Process Biochemistry*, 36(4), 297–303. [https://doi.org/10.1016/S0032-9592\(00\)00153-9](https://doi.org/10.1016/S0032-9592(00)00153-9)
- Wang, S., Ghimirea, N., Xinb, G., Jankaa, E., & Bakkea, R. (2017). Efficient high strength petrochemical wastewater treatment in a hybrid vertical anaerobic biofilm (HyVAB) reactor: A pilot study. *Water Practice & Technology*, 12(3), 501–513. <https://doi.org/10.2166/wpt.2017.051>
- Wang, S., Savvaa, I., & Bakke, R. (2019). A full-scale hybrid vertical anaerobic and aerobic biofilm wastewater treatment system: case study. *Water Practice & Technology*, 14(1), 189–197. <https://doi.org/10.2166/wpt.2018.123>

- Wollmann, F., Dietze, S., Ackermann, J. U., Bley, T., Walther, T., Steingroewer, J., & Krujatz, F. (2019). Microalgae wastewater treatment: Biological and technological approaches. *Engineering in Life Sciences*, 19, 860–871. <https://doi.org/10.1002/elsc.201900071>
- Zaffaroni, C., Daigger, G., Nicol, P., & Lee, T. W. (2016). Wastewater treatment challenges faced by the petrochemical and refinery industry, and opportunities for water reuse. *Water Practice & Technology*, 11(1), 104–117. <https://doi.org/10.2166/wpt.2016.012>
- Zhang, B., & Gao, T. (2000). An anoxic/anaerobic/aerobic process for the removal of nitrogen and phosphorus from wastewater. *Journal of Environmental Science and Health*, 35(10), 1797–1801. <https://doi.org/10.1080/10934520009377075>