Appraisal of Suspended Growth Process for Treatment of Mixture of Simulated Petroleum, Textile, Domestic, Agriculture and Pharmaceutical Wastewater

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Abstract

The unrestricted discharge of domestic and industrial wastewaters along with agricultural runoff water into the environment as *mixed-wastewater* pose serious threat to freshwater

resources in many countries. Mixed-wastewater pollution is a common phenomenon in the developing countries as the technologies to treat the individual waste streams at source are lacking due to high operational and maintenance costs. Therefore, the need to explore the potential of suspended growth process (SGP) which is a well-established process technology for biological wastewater treatment, is the focus of this paper. Different wastewater constituents: representing domestic, pharmaceutical, textile, petroleum, and agricultural runoff were synthesized as a representative of mixed-wastewater and treated in two semi-continuous bioreactors (R1 & R2) operated at constant operating conditions vis., MLSS (mg/L): 4640-R1, 4440-R2, SRT: 21-d, HRT: 48 - 72-h, and uncontrolled pH. The system attained stable condition in day 97, with average COD, BOD and TSS reduction as 84.5, 86.2, and 72.2% for R1; and 85.1, 87.9, and 75.1% for R2 respectively. Phosphate removal on average was by 74.3% in R1 and 76.6% in R2, while average nitrification achieved in systems 1 and 2 were 56.8 and 54.7% respectively. The biological treatment system has shown potential for improving the quality of mixed-wastewater to the state where reuse may be considered and tertiary treatment can be employed to polish the effluent quality.

Keywords: Mixed-wastewater treatment; suspended growth process; Industrial wastewater; Domestic wastewater; agricultural runoff.

1. Introduction

Freshwater pollution is becoming a serious global issue following the rate of influx of organic and emerging pollutants into the environment. The pollution arises mainly via discharges from domestic, industrial, and agricultural activities with adverse impact on public health and aquatic ecosystem [1,2]. With the escalating population growth, industrial expansion and intensified agriculture, an unprecedented degree of freshwater deterioration is unavoidable if proportionate measures are not taken [3]. Unlike in developed world where the standard practice of treating wastewaters at source before disposal or possibly reused is upheld,

discharge of waste streams from different sources into the environment is a common practice in the developing countries in which case the various streams intercept downstream and integrate into a “*mixed wastewater*” with attendant pollution impact on water environments. These practices are in most cases unchallenged as the technologies to efficiently treat the wastes at source are lacking due to high operational and maintenance costs. Industries contribute heavily to water quality degradation and those identified with high pollution impact on the environment in developing countries include but not limited to pharmaceutical, oil and gas, textile mill, tanning industry, pulp and paper, and painting and coating operations [2,4]. When the discharges from these operations intercept downstream with domestic and agricultural runoff, accelerated pollution is inevitable. Hence the need to treat the mixed-waste-streams prior to discharge which is the focus of this paper becomes paramount. Regrettably, technologies such as advanced oxidation processes (AOPs), membrane bioreactors (MBRs), and membrane filtration (MF) with proven records of outstanding performance for wastewater treatment are not obtainable especially in developing countries due to their high chemical and energy requirements. Biological treatment process such as suspended growth process (SGP) is very attractive because it is relatively cheap, robust, efficient, and environmentally benign [5]. The system though has been identified as a direct source of greenhouse gasses (GHG) emissions, suitable control mechanisms and managed aeration and process strategies have been found to reduce N₂O considerably [6]. The SGP comprises of biological aerated reactor where specific concentration of biomass of diverse microbial population is maintained in complete mixture with wastewater, and secondary clarifier for solid-liquid separation. The microorganisms utilize the oxygen supplied to the bioreactor to transform biodegradable polluting organic compounds to stable by-products such as carbon dioxide, water, and new biomass [7]. Furthermore, by virtue of biomass characteristics and managed aeration strategy, SGP can be operated for efficient and

simultaneous removal of nutrients and organic compounds in wastewater [8]. SGP has found high application in municipal [9], domestic [10], and industrial [11] wastewater treatments. Specific studies on SGP proved the system viable for removal of some pharmaceutical compounds (PhACs) such as penicillin-G by 92.7% [12], degradation of textile dyes (methylene blue) by 56% [13] and hydrocarbons (diesel fuel) by 69% [11], nutrients and organic carbon [14]. Nonetheless, diclofenac and anti-epileptic carbamazepine are among the PhACs with persistent threat to biodegradation [15].

While the majority of studies on SGP were focused on individual wastewater, and to the best of the authors' knowledge, no study has been carried out on "mixed-wastewater" comprising of domestic, pharmaceutical, textile, petroleum, and agricultural runoff, an obvious scenario in developing countries. It is therefore the aim of the present study to bridge the knowledge gap by studying the potentials of suspended growth process for treatment of mixed-wastewater. The specific objectives include: - to simulate mixed-wastewater: comprising of constituents representing domestic, pharmaceutical, textile, petroleum, and agricultural runoff as mixed-stream, and characterisation of mixed wastewater; and to treat the mixed-wastewater with suspended growth biological process operated at constant aeration regime, HRT, SRT, MLSS, and uncontrolled pH; and evaluate the system potential for mixed-wastewater treatment application.

2. Materials and methods

2.1. Experimental setup

A schematic illustration of the laboratory scale suspended growth process setup used in the present study is shown in Figure 1. The bioreactors (2-nos) consisted of 5 L plastic tanks with 3.5 L working volume (each), and a feeder tank of 10 L capacity. Oxygen was supplied by dual purpose aquarium pumps (Interpet Aquarium Air pump – AP4) at 5 L/min through stone diffusers by which complete aeration and mixing were maintained. The reactors were

mounted in a fume cupboard and operated under the same conditions at room temperature in a semi-continuous mode. For homogeneity of influent, simulated mixed-wastewater used were prepared fresh in the feeder tank and fed at equal rate and intervals to the reactors. The bioreactors were inoculated with activated sludge seeds and wastewater collected from municipal wastewater treatment plant (Thames plant Reading, United Kingdom). Influent, effluent, mixed liquor, and excess sludge samples were taken on regular intervals to investigate the process.

Figure 1 here

2.2. Composition of Simulated Wastewater Mixture

Due to the spatial variability of real wastewater, synthetic mixed wastewater (MWW) was rather used in this study. The MWW comprised of petroleum, textile, pharmaceutical, domestic wastewater, and agricultural runoff. The composition of the simulated MWW used in the study is presented in Table 1. The domestic, textile, and petroleum were simulated following previous studies [16-19]. Among the different therapeutic classes of pharmaceuticals antibiotics, analgesic, and anti-epileptics are of great concern due to their high frequency of use and detection in the environment. On that note, a representative from each group viz; penicillin-G, diclofenac, and carbamazepine respectively were selected. A mixed stock solution was prepared by adding concentrated, but appropriate amounts of compounds to 1.0 L of tap water. The required substrates and nutrients for the microbial activity was provided in the domestic wastewater. All chemicals used were of high purity grade (>90%) and were obtained from Sigma – Aldrich (Gillingham, United Kingdom) except diesel fuel which was obtained from Esso petrol station, Bramley, Surrey, United Kingdom. High phosphorus and ammonium contents were simulated to represent agricultural tail-water from fertilized fields, pasture and range lands. The pH of the synthetic MWW was adjusted to around 7.3 with 33.6 mg/L NaHCO_3 . The stock solution was homogenized in a

mechanical mixer operated at 90 rpm for 1 hr. The solution was preserved at 4°C in a refrigerator, and feed samples to the bioreactors were prepared fresh every 2 days. Analyses (COD, NH₃, and PO₄) performed on stock solution bi-weekly showed that the mixture was stable.

Table 1 here

2.3. *Experimental Operation/procedure*

The reactors were first inoculated with activated sludge seeds (0.5 L) and regular municipal wastewater (2.5 L) and synthetic mixed wastewater (SMWW) (0.5 L), and was then operated for acclimation to SMWW, which was increased step-wisely in the order of 0.5 L every week. The systems were operated under semi-continuous mode at constant operating conditions with treatment and settling times of 48 h and 1 h respectively. The biological units were fed thrice every week with equal volume of effluent. The operating conditions upon which the systems were monitored include constant temperature and aeration, uncontrolled pH, constant MLSS and SRT. Due to the location of the reactors (under a fume cupboard) evaporation was intense and was compensated before effluent discharge. After treatment and settling, clear supernatant effluents were decanted from the systems and same volume of synthetic mixed wastewater was added to the reactors to maintain the 3.5 L working volume. The mixed liquor suspended solids (MLSS) concentration were maintained nearly constant in both units by constant removal of equal volume of excess sludge at regular intervals. Due to some operational constraints, the last weekly feed wastewater were given a 72-h retention. The performance of the system were monitored on effluents with 48-h and 72-h retention times. Effluent samples were analysed for physico-chemical and microbial quality bi-weekly to ensure the systems have attained a steady state.

2.4. Physico-chemical Characterization of Simulated Wastewater Influent and Effluents

The organic matter contents of water samples (influent and effluent) were accessed as 5-day BOD₅ and COD with standard methods (SMs): 5210 B and 5220 D respectively. The concentration of nutrients was measured as phosphorus, ammonium, nitrate and total nitrogen in compliance with the Hach Spectrophotometry methods 8114, 10031, 8039, and 10072 adapted from SMs 4500 P C, 4500 NH₃, 4500 NO₃ E, and 4500 N_{org} B respectively. Total suspended solids was measured gravimetrically in line with SM - 2540 D; turbidity (SM 2130 B) was measured using a portable Hach turbidimeter (Hach: 2100P), electrical conductivity (SM-2510) and temperature (SM: 2550B) were measured using a TDS/EC/Temp probe (Hanna: HI 98360); true colour was measured in line with Hach Spectrophotometry method 8025, adapted from SM-2120 C, pH was measured using a bench-top pH probe (Hanna: HI 2210), and total coliform was analysed following SM: 9922. Mixed liquor suspended solids, mixed liquor volatile suspended solids, dry sludge weight, and sludge volatile suspended solids were determined in line with the standard methods, as according to the standard methods [18].

2.5. Scanning Electron Microscopic (SEM) Study of the Sludge Samples

The surface morphology of the biomass was examined with high resolution scanning electron microscope (SEM) (JSM – 7100F), Department of Mechanical Engineering Sciences, University of Surrey, UK. The sludge samples (seed sludge, biomass in reactor 1 and reactor 2) were pre-treated following the method stated in previous study [19]. 1 ml of sludge sample was introduced into 3 ml of Eppendorf tubes and washed 4 times with distilled water by centrifugation at 13000 rpm for 2 min using (Eppendorf Centrifuge 5415 C). The washed biomass cells were re-suspended in 2 ml distilled water and about 10 µl was spotted on high purity carbon stubs and left to dry in a desiccator for 2 days. For high resolution imaging,

protection of biological samples from the damaging impact of electron beams and material conductivity, the sludge samples were gold coated under vacuum to about 1 mm thickness and loaded on SEM equipment for imaging [19].

3. Results and discussion

3.1. Results

3.1.1. Physico-chemical Characterisation of Synthetic mixed wastewater

Table 2 reports the average values of physico-chemical and microbiological characteristics of the simulated mixed wastewater. The BOD₅/COD ratio of the mixed wastewater was 0.13, and this signifies a non-biodegradable mix which has to do with its chemical origin. The high nutrient contents in the mix simulates agricultural tail-water and runoff from grazed field.

Table 2 here

3.1.2. Removal of Organic matter and nutrients

Due to the limitations of the present study, the aggregate compositions of PhACs, hydrocarbons and MB in treated effluent were not quantified. Data relating to organic carbon and nutrient removal are shown in the Figures 2 and 3 respectively. While phases A and B of the figures represent acclimation stage and biological activity to steady-state respectively, phase C represents the system performance at 72-h retention time in steady-state. Average residual COD and BOD₅ in the treated effluent during acclimation were 101.7±32.3 and 25.9±5.5 mg/L for reactor 1; while reactor 2 had 104.8±33.5 and 29.2±6.3 mg/L respectively. After full acclimation that lasted for 45-d, the systems underwent varying biological activity before arriving a stable state on day 97. The average organic carbon contents at steady-state (Fig 2 a & b, phase B) were 221.6±34.2 mg COD/L and 25.3±10.5 mg BOD/L for bioreactor 1; 213.4±36.4 mg COD/L and 22.3±5.6 mg BOD₅/L for bioreactor 2.

Ammonium oxidation was nearly limited at start-up and became active at the tail of acclimation period with average reduction as 29.4 ± 4.1 and 29.1 ± 4.8 mg/L for treatment units 1 and 2 respectively. Upon growth of nitrifying bacteria and full acclimation, nitrification resumed actively in the biological systems (Fig 3a, phase B) leading to a reduction in $\text{NH}_4\text{-N}$ concentration to an average of 20.0 ± 4.0 and 20.9 ± 3.8 mg/L at steady-state in both reactors respectively. Ammonium consumption was in tandem with nitrate production in the treatment units (Fig 3b). From the point nitrification resumed appreciably, the concentration of nitrate increased and stabilized at an average of 109.1 ± 17.0 mg $\text{NO}_3\text{/L}$ in reactor 1 and 103.4 ± 16.3 mg $\text{NO}_3\text{/L}$ in reactor 2. Average phosphate concentration in treated effluent at steady-state for reactors 1 and 2 were 25.5 ± 5.9 and 23.2 ± 6.6 mg/L respectively. The total nitrogen concentration (Fig 4a) decreased from 142 mg/L to an average of 66.0 ± 10.8 mg/L at steady-state in both units.

3.1.3. pH profile

The pH of the system was not controlled and hence varied significantly (Fig 2c) switching between weak acidity and alkalinity, before maintaining a near stable neutral condition. The average pH at steady-state was 7.0 ± 0.3 .

Figure 2 here

Figure 3 here

3.1.4. Other physico-chemical parameters

Figures 4b & 4c present the results for total suspended solids, turbidity and Figure 5a shows the result for true colour. The solids in the treated effluents first decreased abruptly at start-up due to dilution effect of the inoculum wastewater, and later risen to 69 ± 18.8 TSS mg/L in reactor 1 and 78 ± 43.3 TSS mg/L in reactor 2 during acclimation in response to increased feed dosage. Similarly, turbidity increased to 94.0 ± 29.2 and 97.4 ± 34.2 NTU after the dilution

effect in reactors 1 and 2 respectively. At steady-state, the systems achieved an average TSS removal of 72.2 and 75.1% for reactors 1 and 2; while turbidity removal were 86.6 and 88.7% respectively. Residual true colour was almost undetectable at steady-state. Table 3 shows the results of electrical conductivity (EC) and total dissolved solids (TDS) in both systems at acclimation and steady-state. EC and TDS increased sharply from 982 ± 1.2 μS and 491 ± 1.0 mg/L as average feed concentration and stabilized at an average concentration of 1009.4 ± 75.8 μS and 504.6 ± 38.0 mg/L ; and 976.6 ± 77.2 μS and 482.6 ± 43.2 mg/L for reactors 1 and 2 respectively.

Figure 4 here

Table 3 here

3.1.5. Microbial count

Total coliforms were monitored to assess the ability of the treatment process to effectively remove faecal bacteria present in wastewater environment. Results for total coliform counts are presented in Figure 5b. Total coliforms increased gradually and inconsistently from start-up before attaining maximum values of 4.1×10^5 CFU/100 ml and 5.0×10^5 CFU/100 ml in reactors 1 and 2 respectively. Later, the concentration decreased and stabilized at average values of 2.53×10^4 and 1.24×10^4 CFU/100ml at steady-state in the two systems respectively.

Figure 5 here

3.1.6. Sludge morphology and community

The morphology of the seed sludge and biomass after acclimation (day 63) and steady-state (day 97) are presented in Figure 6.

Figure 6 here

3.2. Discussion

The suspended growth system consisted of a mixture of biomass flocs and aerobic granules was acclimatized in 45 days. During the operation, a near stable F/M ratio of 0.05 d^{-1} were maintained in the bioreactors which correlated with the operated SRT of 21 d. However, endogenous respiration also contributed to the near constant F/M ratio [20]. This value is within the range recommended for activated sludge process with nitrification [21] and extended aeration [24,25]. The MLSS concentration in the systems (reactors 1 and 2) did not vary significantly but remained at average values of 4440 and 4640 mg/L respectively, with uniform MLVSS of 2460 mg/L average. The MLVSS only varied temporally between day 51 and 91 with the concentration in reactor 2 about 40 mg/L more than the concentration in reactor 1. The MLVSS concentration employed in the present study is similar to the ones used in related investigation [24] and treatment of mixed petroleum refinery and domestic wastewater [20]. The ratio of MLVSS/MLSS in both bioreactors were in the range of 0.53 - 0.55 which indicates high content of inorganics in the mixed units. However, the high concentration of MLSS in reactor 1 was due to more precipitation of inorganic (sediments) in the system which was suspected to have caused by the higher evaporation rate from the treatment unit. Considering the relevance of MLSS in wastewater treatment, as too low concentration will lead to poor treatment and very high values will amount to self-degradation of microorganisms due to insufficient food [25]. The concentration used in the present study was maintained relative to the recommended range (1500 – 4000 mg/L) for completely mixed suspended growth biological process for organic carbon and ammonium removal [7].

3.2.1. Removal of Carbonaceous Pollutants

The drastic decrease in effluent COD, and BOD_5 at start-up was mainly due to dilution effect by seed municipal wastewater. However, the performance of the reactors were monitored

after a phase-out of the dilution effect achieved after feeding the reactors more than twice the working volume [26]. The average COD and BOD₅ removal during acclimation were 92.90 and 85.89% for reactor 1, and 92.68 and 84.65% for reactor 2 respectively. The high organic matter removal suggests the activeness and dominance of the heterotrophic bacteria. During the adaptation process, the microbial cells received some varying levels of shock in response to increased feed dosage and toxicity impact, which reflected on the effluent quality. The sinusoidal trajectories exhibited in oxidation of carbonaceous matter (Fig 2 a & b) are common phenomena associated with microbial acclimatizing to foreign environmental conditions [27]. Phase-A of Figure 2 a & b depict microbial activity with quick adaptation to the mixed wastewater conditions and short resuscitation from shock loads, which explains the robustness of the heterotrophic bacteria.

The biological system reached pseudo steady-state in day 97 after a consistent plant operation. Phase-B of Figure 2 a & b present the trends of COD and BOD₅ removal after adaptation to wastewater conditions. The biomass in the aeration tanks exhibited some varying degrees of bioactivity which became apparent from day 72 and later came into synch at steady-state. The high treatability performance in reactor 2 could be a product of higher microbial population which reflected on the MLVSS concentration within the time range. Oxidation of carbonaceous matter in similar studies was found to have a direct relationship with biomass concentration with higher COD removal at increased MLVSS concentration and higher HRT of 36-h [28]. The average COD and BOD₅ removal in reactors 1 and 2 at steady-state were 91.69 and 86.22%; and 90.04 and 87.85% respectively. Similar efficiencies have been reported for biodegradation of organic matter in mixed industrial and domestic/municipal wastewater using well adapted bacterial strains [29] and activated sludge biomass [20].

3.2.2. Removal of Nutrients

297 Ammonium oxidizing bacteria (AOB) had earlier shown a rather near stable condition (Fig
298 3a, phase-A) after overcoming a one-off flash shock which occurred at dose transition phase.
299 The near constant trend dynamics in nitrification in both bioreactors illustrated the
300 characteristics of slowly growing organisms which implies a near stable autotrophic bacteria
301 population [32,33]. Oxidation of ammonia resumed appreciably on day 28 after increased
302 number of nitrifying organisms with average efficiency at acclimation stage as 36.5 and
303 37.0% for bioreactors 1 and 2 respectively.

304 The increase in residual nitrate (Fig 3b, phase-A) in treated effluent at start-up period could
305 be from seed municipal wastewater as the nitrification pathway was limited due to low
306 bacteria population. The steady reduction in nitrate which ensued at limited nitrification
307 phase implies denitrification of the near constant residual (original) nitrate believed to have
308 occurred within the anaerobic environment of aerobic granules and flocs created due to
309 limited air diffusion in the biomass structure [34,35,36]. The sudden increase in nitrate
310 concentration in treated effluent from day 28 was in tandem with the nitrification activity
311 shown in Figure 3a, phase-A. Upon full growth of autotrophic bacteria and good acclimation
312 to mixed wastewater conditions, nitrification became very effective and maintained a
313 downward sinusoidal dynamic in both systems (Fig 3a, phase-B), although, there was a
314 considerable and temporal variation in ammonia consumption between the bioreactors.

315 The difference in nitrification rate could be due to biomass inequality in both systems as well
316 as some unavoidable natural phenomena. This observation is in corroboration with nitrate
317 production in the systems (Fig 3b, phase B). The average nitrification achieved in reactors 1
318 and 2 at steady-state were 56.8 and 54.7% respectively. These results are consistent with
319 other related study [35] but in disagreement with [10] who achieved a complete nitrification
320 and 90% phosphate removal at constant high aeration. The variation in results could be due to
321 the difference in biomass concentration and length of operation in the two studies as well as

the characteristics of the mixed wastewater used in the present study. A group of researchers, attributed the low ammonium removal (61%) in their work to low dissolved oxygen concentration [35]. In the present study, DO was not a limiting factor for oxidation of ammonium nitrogen since dissolved oxygen concentration of more than 5 mg/L was maintained throughout the operation. The observed low nitrification could be linked to temperature effects as the systems were operated at an average temperature of 19.5°C which was below the optimum range (28 and 36°C) for plateau of maximum nitrifying activity [36]. Additionally, HRT may have contributed to the observed low nitrification in the treatment units. This is apparent with the near stable sinusoidal pattern exhibited at steady-state phase (Fig 3a, phase B) with the valleys and peaks representing ammonia consumption at 72 and 48-h contact times respectively. Further evidence is shown in phase C where a near constant nitrification was maintained at 72-h retention time. This finding corroborate with the results of [37] who reported that a decreased in HRT from 30 to 5 h was accompanied by a 55% bacteria reduction with resultant low nitrification activity. Another factor that may have contributed to the relatively low nitrification in the system is pH. An average value of 7.0 were measured in the reactors at steady-state and which is below the range (7.3 – 8.0) reported for efficient and stable nitrification [38]. Before reaching the steady-state, nitrification was more efficient in reactor 1 than in reactor 2. The observed variation may be due to bacteria inequality in the systems which reflected on MLVSS concentration between day 51 and 91.

After well acclimation to wastewater condition, nitrate production gradually increased in reactor A and later decreased and stabilized at steady-state, while in reactor 2, there was a short increase in residual nitrate which decreased afterwards before attaining a stable state at higher concentration and became in synch with reactor 1 (Fig 3b). As the systems were operated under continuous aeration regime which favoured the nitrifying bacteria, there was a

build-up of nitrate in the treated effluent which amounted to average production of 81% at steady-state in both reactors. The observed trends of nitrate concentration in both systems are in corroboration with the ammonia consumption dynamics shown in Figure 3a) with the valleys representing points with higher nitrification activity and vice versa. Again, there were some denitrifying phosphate accumulating organism process which occurred within the biomass floc structure due to limited oxygen diffusion, thus leading to an average 55.6% denitrification in both bioreactors measured in terms of TN removal at steady-state condition. This result is similar to the one reported by other researchers [39]. The observed denitrification in the aerobic systems may have occurred in anaerobic environment created under localised oxygen depletion within the biomass granules and floc structure [33].

Similarly, seed municipal wastewater was responsible for the sudden decrease in phosphate concentration in treated effluent which gradually increased in response to increased feed dosage (Fig 3c, phase-A). The subsequent gradual reduction in phosphate in the aerated bioreactors could be by denitrifying phosphate accumulating organisms process [34,42,43]. The phosphate uptake mechanism (Fig 3c, phase-A) during acclimation follow similar trend with nitrate profile shown in figure 3b, phase-A. Similar relationship have been reported in previous works [42,44], although their studies were conducted in required redox conditions, but this phenomenon may have occurred in limited air diffused zones of aerobic granular sludge and flocs [32] used in the present study. The average phosphate removal in both units during acclimation period was 84%.

After acclimation, phosphate uptake rate reduced to an average value of 74.3 and 76.6% at steady-state in reactors 1 and 2 respectively. The observed reduction was due to limited anaerobic conditions and build-up of phosphate in the treatment systems. These results disagreed with [14] who reported a 56% phosphate uptake in a complete aerated Modified Ludzack-Ettinger process under carbon limited condition. The high phosphate removal in

suspended growth system could follow different pathways. One of which is denitrifying phosphate accumulating organisms process [32,41] occurring inside the anaerobic zone of aerobic granules, though this process was limited due to limited anaerobic conditions and there were more of flocs than granules in the biomass. Another pathway for phosphorus removal is by calcium phosphate precipitation [43]. In the present study, phosphate may have been removed by calcium phosphate precipitation into the particulate matter thus contributing to the concentration of suspended particles measured in the treated effluent. This could be very possible as the trend dynamics of residual phosphate follow similar pattern with that of suspended solids measured in terms of turbidity and TSS. Moreover, iron and aluminium compounds which was present in low concentration (EDX analysis: results not shown) may have contributed to phosphate precipitation in the aerobic treatment system [43]. Finally, organic sludge wasting may also be responsible for the observed reduction in phosphorus removal [43].

3.2.3. pH profile

The pH of the system was monitored periodically and the profile from start-up to steady-state is presented in Figure 2c. The profile shows a considerable reduction in pH from 7.3 to an average of 6.24 in both reactors at start-up. The observed fall was the effect of seed wastewater used for inoculation. Overtime, the system regained alkalinity which was subsequently consumed at the phase of effective nitrification as about 8.64 mg of HCO_3^- is reported to be utilized for every mg of ammonia nitrogen oxidized to nitrate [38]. The later decrease in pH was due to the formation of hydrogen ions in the solution during ammonium oxidation to nitrite. This result is in line with the findings of previous studies [40,46,47]. The biological system regained alkalinity afterwards from day 60 to 77 and this could be due to neutralization of hydrogen ions by bicarbonate ions in the solution which resulted to CO_2 stripping [38,44]. As nitrification continued and became very effective up to steady-state,

there were considerable reduction in pH which signifies efficient nitrifying activity. In earlier work [38] it was stated that about 8.64 mg of alkalinity (HCO_3^-) are consumed for ammonia oxidation to nitrate which cumulatively affect pH stability. Thus, the further variations in pH in the present study could be aligned to the stated phenomenon.

3.2.4. Other physio-chemical parameters

The solids in the treated effluents increased with increased feed dosage and attained stability at steady-state phase (Fig 4 b & c). The maximum TSS removal at steady-state were 72.2 and 75.1% for reactors 1 and 2, while turbidity removal was 86.6 and 88.7% respectively. The treated effluent contained more of colloidal particles which reflected on the measured higher residual turbidity over the total suspended solids. However, the concentration of residual turbidity in treated effluent (Fig 4c) follow similar trend with the total suspended solid (Fig 4b) which implies that turbidity measurement can be employed as a surrogate for monitoring total suspended solids dynamics in water systems. This finding agrees with the results published in other work [46]. The variation in residual apparent colour (data not shown) was in synch with suspended solids. This implies that the molecules of methylene blue dye have strong affinity for suspended particles simulated with kaolin clay in this study, thus suggesting high sorption potentials of basic dyes. Similar findings were reported by Khattri and Sing [47] who found clay materials as good adsorbent for colour removal. Filtering the effluent through 0.45-micron filter paper, residual true colour (Fig 5a) became almost undetectable at steady-state. This results strongly corroborate with the high sorptivity of methylene blue dye as reported in previous work [48].

EC and TDS in both systems increased sharply from $491 \pm 1.0 \mu\text{S}$ and $982 \pm 1.2 \text{ mg/L}$ average feed concentration to $1336.2 \pm 11.5 \mu\text{S}$ and $668 \pm 9.9 \text{ mg/L}$ at start-up and later stabilized to an average concentration of $993 \pm 23.2 \mu\text{S}$ and $493.6 \pm 15.5 \text{ mg/L}$ at steady-state (see Table 3). The sharp rise in measured parameters at start-up aroused partly from the seed municipal

wastewater and increased feed dosage. Total dissolved solids concentration have been found to have a negative impact on the performance of ASP in terms of organic matter and nutrients removal by interfering the oxygen transfer necessary for biological metabolism [49,50]. Wu and co-worker reported that TDS concentrations below 1500 mg/L have no significant impact on COD and phosphate removal and values above could seriously stymie the aerobic metabolism of the system. As the TDS concentration in the present study was about 67% below the threshold (1500 mg/L), it is expected that it had not affected the system performance significantly.

3.2.5. Microbial count

Total coliforms (TC) were monitored to assess the ability of the treatment process to effectively remove faecal bacteria present in wastewater environment. The presence of coliform bacteria in the treatment units (Fig 5b) sprang from the municipal wastewater used for inoculation as the wastewater was synthesised and had no faecal bacteria. Total coliforms increased inconsistently to 4.1×10^5 and 5.0×10^5 CFU/100ml in reactors 1 and 2 from start-up and gradually decreased to average values of 2.53×10^4 and 1.24×10^4 CFU/100ml at steady-state respectively. These values are greater than the permissible limits of less than 1000 CFU/100ml recommended by WHO for unrestricted irrigation of edible crops [51]. The spatial variation in coliform bacterial concentration (Fig 5b) could be due to changes in suspended solids concentration as reflected on the measured TSS and turbidity. Similar relationship has been reported by other researchers [52]. Although complete inactivation was not expected, but the systems achieved considerable high reduction in TC at steady-state at low residual suspended solids concentration. The measured reduction are in coherence with the findings of earlier studies [53,54]. Following the considerable reduction in TC at lower suspended particles concentration, solids separation treatment and subsequent disinfection are most advised for improved effluent quality.

3.2.6. Organic carbon and phosphate variation with suspended solids and Hydraulic retention time (HRT)

Residual organic carbon and phosphate in treated effluent varied relatively with suspended solids and inversely with retention time which therefore suggests the presence of slowly biodegradable and un-settle-able organic matter in the treatment units. As the systems were fed thrice every week at constant retention time of 48-h, due to some operational constraints, the last weekly feeds were given a 72-h retention time. At this higher contact time, more particulate matter was found to precipitate out of the solution which resulted in higher COD and phosphate removal efficiencies observed at the valleys of the removal trends presented in phase B of Figures 2a and 3c respectively. The sinusoidal dynamics exhibited at steady-state is in tandem with retention time (HRT) as the system performance was monitored on effluent with 48 and 72 hr contact times. However, further system assessment at 72-h contact time (phase C) got the sinusoidal dynamics into extinction. As was previously observed [55], a reduction in HRT by 14 hr led to about 6% reduction in COD and BOD removal in similar wastewater treatment units. In another study [24], a significant reduction in BOD removal (22%) was observed when HRT was reduced from 12 to 4 h and COD removal was reduced from 91 to 77% when HRT was decreased by 18 hrs [28]. Following the observed relationship between the suspended solids (measured as turbidity and TSS), organic matter, and phosphate concentration in the treatment systems, extended HRT will no doubt enhance further precipitation of slowly and un-settle-able biodegradable matter.

3.2.7. Variation between the unit systems

Prior to attainment of steady-state, there was a temporal variation in bacterial activity between the two treatment units from day 51 to day 91 which led to higher treatment efficiency in reactor 2. It is obvious that biomass concentration may have played a great role in the observed development as the MLVSS was higher in reactor 2 within the referenced

time. Previous studies have reported similar observation. However, the higher nitrification in reactor 1 implies that the excess biomass between days 51 and 91 were mainly heterotrophic populated. On arriving a stable condition, the active biomass in both units became relatively the same, thereby maintaining uniform dynamic activity. The initial shift (increase) in microbial population in reactor 2 could be matched to unavoidable natural process, and the subsequent population reduction may be an effect of self-degradation of microorganisms due to insufficient food [25].

3.2.8. Sludge morphology and community

The seed sludge and biomass in reactors 1 and 2 were taken to observe the dynamics of the sludge morphology. SEM images showed that the seed sludge (Fig 4.5a) was loosely populated by various bacterial micro-colonies mainly coccus, rod-shaped and filamentous-shaped bacteria. Such morphological structures of inoculum sludge have been used for treatment of synthetic wastewater [56]. After acclimation (day 63) the biomass in the two reactors became densely and multifariously populated by round and both short and long rod-shaped cells covered with exopolysaccharides (EPSs). It implies that during acclimation the microorganisms became well adapted to the conditions of the mixed wastewater, hence became less vulnerable to toxic shock and multiplied. Nevertheless, the EPSs which developed on the biomass structure may have played a vital role in shading the microbial cells against toxic impact of the mixed wastewater. Similar bacterial colonies have been reported to be dominant in activated sludge system treating domestic wastewater [44] and textile wastewater [13,57].

4. Process analysis

Figure 7 presents the flow diagram of the single unit suspended growth process used for the treatment of mixed-wastewater. The overall system operating conditions are presented in

Table 4. The biomass concentration in the reactors were kept nearly constant throughout the process by maintaining a regular withdrawal of excess sludge from the system, thus sludge accumulation was negligible. The mass balance of solids in the bioreactors is presented as follows;

$$Inflow_{solids} - Outflow_{solids} = Accumulation = 0 \quad (1)$$

$$(Q_{in} + Q_R)X = Q_{r1}X_r + Q_{r2}X_s + Q_wX_r + Q_sX_s + Q_xX \quad (2)$$

The bioreactor operating parameters, hydraulic and sludge retention times (HRT and SRT) were determined from the following expressions;

$$HRT = \frac{V}{Q} \quad (3)$$

$$SRT = \frac{VX}{Q_wX_r + Q_sX_s + Q_wX} \quad (4)$$

Where; Q = inflow rate (L/d); V = effective volume of reactor (L); X_e (concentration of solids in effluent) is negligible = 0; other terms are as defined in Figure 7.

Figure 7 here

Table 4 here

The biological process indicators are shown in Table 4. The biological process was assumed to be under steady state for the assessment of the process. The HRT (48 h) of the system can be considered on the high side. However, the system was designed and operated to ensure that the HRT is not the limiting parameter for the efficiency of the biological process. Similarly, the SRT (21 days) of the biological process was also on the high side, which ensures that SRT was also not the limiting parameter for the growth of slow-growing micro-organisms such as nitrifying bacteria. Therefore, the performance of the biological process

provides a realistic and achievable process efficiency of the biological process in terms of the removal of organic pollutants present in mixed wastewater.

5. Conclusion

The experimental results obtained from the suspended growth process indicate the presence of diverse microbial community and proved the system robust and viable for treatment of simulated mixed-wastewater considered in this study. While its performance at varying operating conditions and on micro and organic pollutants removal are under study, the system attainable efficiency at steady-state operation and constant aeration regime is summarised as follows;

- Average BOD and COD reduction by 87.0 and 84.8% respectively.
- Ammonium consumption by 56.8 and 54.7% for bioreactors 1 and 2 respectively.
- Phosphate removal by 74.3 and 76.6% for both units.
- 72.2 and 75.1% TSS removal for reactor 1 and 2 respectively.
- TC reduction from average of 4.1×10^5 and 5.0×10^5 CFU/100ml in reactor 1 and 2 to 2.53×10^4 and 1.24×10^4 CFU/100ml.

Though the treated effluent quality may not be considered suitable for discharge into the aquatic environment or for reuse applications directly. However, the system has significantly improved the wastewater quality to a state, that its reuse may be considered for specific situations. Hence, the potential reuse of the treated wastewater for irrigation of edible crops should be investigated to determine effluent reusability and identify the threshold quality for such application. Further post treatment may be required for polishing purposes to increase the applicability of the reclaimed wastewater for unrestricted irrigation which can recover freshwater resources from further depletion and pollution.

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Conflict of interest

The authors declare that they have no conflicting interests.

Ethical approval

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

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Legend for figures

Figure 1. A schematic illustration of the experimental setup employed for the treatment of Mixture of Wastewaters

Figure 2. Performance of bioreactors at startup acclimation to steady state condition with respect to removal of (a) COD (b) BOD₅ and (c) pH variations

Figure 3. Nutrients removal at startup acclimation to steady state condition with respect to (a) Ammonium (NH₄), (b) Nitrate (NO₃) and (c) Phosphorus (PO₄).

Figure 4. Performance of bioreactors with respect to (a) Total nitrogen (b) Total suspended solids (TSS) and (c) Turbidity.

Figure 5. Results of treated effluent parameters with respect to (a) True colour (b) Total coliform.

Figure 6. Scanning Electron Micrographs (SEM) for the sludge samples (a) seed sludge (b) day 63 RA (c) day 63 RB (d) day 97 RA and (e) day 97 RB.

Figure 7. Suspended growth process flow diagram. Q_{in} = Influent (feed) flow rate; Q_x = Flow rate from reactor for mixed liquor suspended solids determination; Q_e = Effluent flow rate; Q_w = Waste sludge flow rate; Q_{r2} = Return effluent flow rate; Q_{r1} = Return sludge flow rate; X = Mixed liquor suspended solids; X_e = concentration of solids in effluent; X_r = Return sludge concentration; $Q_R = Q_{r1} + Q_{r2}$; V = Volume of reactor (L). All Q are in L/d; All X are in mg/L.

Legend for Tables

Table 1. Composition of synthetic mixed wastewater (MWW) used in experiments

Table 2. Characteristics of synthetic mixed wastewater used in the present research experiments

739 **Table 3.** Chemical characteristics of effluent from June to December, 2018.

740 **Table 4.** Bioreactor operating parameters