

HYBRID MICROALGAL-ACTIVATED SLUDGE TREATMENT PROSPECTS: A REVIEW

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ABSTRACT

Enormous amount of wastewater of varying strength and contamination is produced due to domestic, municipal, agricultural, and industrial activities. These usually contained a high level of pollutants including inorganic and organic pollutants, pesticides, and heavy metals. Physio-chemical treatment approaches were employed to treat these recalcitrant contaminants and effectively handle extremely toxic substances within a short period. However, despite this, these treatments were associated with setbacks, including incomplete pollutant removals, lack of multiple pollutants removal capabilities, excessive sludge production, and cost of energy and chemical employed. Hybrid microbial treatment systems were efficient due to their potential to remove multiple contaminants like heavy metals, BOD, COD, assimilate nutrients, production of renewable energy alternatives, and easily recycled or regenerated. The future bottleneck in terms of best microbial proportions for higher growth rates and removal efficiencies is highlighted.

Keywords: algae, bacteria, proportion, heavy metals, adsorption, toxic

INTRODUCTION

In his arduous search to meet desires and aspirations for better well-being, man produces a wide variety of waste substances that are indirectly or directly released into the surroundings that accumulate and lead to several environmental problems with detrimental consequences [1]. A considerable amount of wastewater was produced due to domestic, municipal, agricultural, and industrial activities of varying strength and contamination [2]-[4]. These usually contained a high level of pollutants that include inorganic and organic pollutants, pesticides, and heavy metals.

Excessive levels on biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), phenols, arsenic, oils, nutrients, colours, and fats were identified within these effluents [5]-[7] and on the other hand, heavy metals like chromium (Cr), lead (Pb), nickel (Ni), iron (Fe), copper (Cu) zinc (Zn) and many

more were reported from industrial effluents [8]-[11]. These contaminants were found to be teratogenic [12], mutagenic [13], and carcinogenic [14] even at trace concentration.

Physio-chemical Treatment Approach

Heavy metals containing effluent were produced due to industrial processes that comprise tanning, dying, mining, metal plating, smelting, and production of batteries, textile, ceramics, and glass [8]. These metal ions were highly toxic, persistent, and mobile even at a lower concentration. Prolonged exposure to these heavy metals may lead to nasal septum perforation, larynx, and liver inflammation, skin allergies, dermatitis [15]. This was also linked to the distorting the formation of protein, cytoplasmic molecules, and DNA in the cell [16].

Due to the vast amount of wastewater produced daily and its recalcitrant effect on lives and the environment, several physico-chemical and biological treatment methods were employed to treat the

contaminants to non-toxic or less toxic substances. These treatment approaches include sedimentation [17], flocculation, coagulation [7],[18], precipitation [19]-[20], electrocoagulation [21], activated sludge process [14],[22], adsorption [7],[17],[20],[23],[24],[25], [26]-[30], using bacteria [31], fungi [9],[32] as well as treatment with microalgae [10]. Table 1 summarises

the physio-chemical treatment approach employed to handle various wastewater generated.

Sequel to the results presented in Table 1, it could be understood that the physiochemical treatment methods performed excellently in the removal of organic and inorganic contaminants that ranges

Table 1 Some wastewater treatment systems

Treatment	Finding(s)	Reference
Chemical Precipitation using Ferrous Sulphate at pH of 8.7	Chromate concentration was reduced from 30 mgL ⁻¹ to 0.01 mgL ⁻¹	[19]
Precipitation using NaOH	Cr(III) was coordinated with hydroxyl or carbonyl functional groups	[20]
Chemical Coagulation (CC) and Electrocoagulation (EC)	Al is more suitable electrode in EC due to Al ³⁺ specie for pollutant destabilization	[33]
Electrocoagulation using Fe-Fe Electrodes	Maximum removal efficiencies of COD, TDS, pH, Colour, and Chloride was found at 0.25 A at 20 min	[21]
Coagulation using Iron (III) chloride (FeCl ₃)	Best removal efficiencies at coagulant dose of 150 mgL ⁻¹	[7]
Coagulation and flocculation in SBR	Removal of 80, 82, and 99.6% of COD, TN, and NH ₄ ⁺ -N, respectively	[18]
Coagulation and Sedimentation in MBR	Organic substance and nitrogen removal was 81 and 36%	[17]
Activated sludge Process at 3500 mgL ⁻¹ MLVSS	BOD and COD removal efficiencies to be 90 and 80%	[22]
Couple Activated Sludge and Domestic Wastewater	Removal of BOD, COD, NH ₄ ⁺ N, NO ₃ ⁻ -N, PO ₄ ³⁻ -P, Ni, Cu, Zn, and Cr as 90.8, 80.7, 98.9, 89.6, 90, 99.5, 92.2, 54.5, and 56.3%, respectively.	[14]
Cr(VI) adsorption using formaldehyde-treated saw-dust and charcoal of sugarcane bagasse at a ratio of 1:4 (Saw-dust: Formaldehyde)	Removal of Cr(VI) was found to increase with the initial increase of concentration	[25]
Synthesis of 2-hydroxyethyl-trimethyl ammonium chloride (HGCTS) for Cr(VI) adsorption	The maximum adsorption capacity was 250 mgL ⁻¹ , and the detection limit was 20 mgL ⁻¹	[10],[24]
Settling, filtration using sand-stone and saw-dust and then coagulation (FeCl ₃) from 50-500 mg/L	150 mgL ⁻¹ coagulant dose with a pH near neutral provide the highest treatment efficiency though with a high TDS above 4000 mgL ⁻¹	[7]
Domestic wastewater treatment in an intermittent aeration reactor followed by distilled-air-floatation	The removal efficiency are COD = 92%, P = 90%, PO ₄ ³⁻ = 84% for raw samples and 94% for filtered samples (filter: 0.45 µm)	[27]
100 µg/g of zeolite in Cr(VI) removal	About 80 µg/g of Cr(VI) was adsorbed from dichromate solution	[5],[28]
Production of AC from granulated shell	The external surfaces of the chemically activated carbon are full of cavities but associated with excessive use of chemicals for impregnation and energy up to 1000°C for carbonization	[23]
Production of AC from Nigerian bamboo tree and subsequent heavy metal removal	Removal efficiency in order of Cd ²⁺ > Zn ²⁺ > Pb ²⁺ > Ni ²⁺ > Cu ²⁺ > Cr ³⁺	[30]
Alkalis precipitation and Cr speciation	Cr concentration > 5000 mgL ⁻¹ can be reduced to about 20 mgL ⁻¹ , and Cr(III) complexation was established	[20]

Cont.

Treatment	Finding(s)	Reference
Adsorption of Cr(VI) using calcium phosphate	Maximum adsorption attained within the range of 1.0-5.0 pH	[34]
Tannery effluent treatment by sedimentation, dissolved air floatation, membrane bioreactors (MBR) and then polishing using granular activated carbon	81% removal efficiency by MBR for organic substances and total nitrogen removal of 36% was recorded	[17]
Adsorption using leather shaving (LS) and goat hair (GH) AC	Decrease of chromium by 78%-73% for the activated carbons prepared from (LS) and (GH), respectively	[26]
Wood-based powdered activated carbon for Cr(VI) removal	The highest Cr(VI) removal was 40.04% at a pH of 3.0, while higher pH of 10.0, the removal efficiency decreased to as low as 0.34%, and 78.8% of Cr(III) was adsorbed	[6]
Kaolin is a natural adsorbent in pollutants removal from tannery wastewater	Lower kaolin doses influenced the ionic competition among the metal ions due to small surface area and increases as the dosage increase, and 72.29, 87.81, and 70.75% of Cr, Cd, and Zn was removed, respectively	[17],[35]
Tannery wastewater treatment using Koalin/ ZnO as nano adsorbents	Maximum removal of Cr(VI), Fe, COD, BOD, and Cl as 100, 98, 95, 94, and 91%, respectively, after series of treatments	[36]
Tannery wastewater treatment using electrocoagulation with Fe electrodes	Optimum Cr removal at a current density of 15 mA/cm ² and a high initial Cr concentration with a pH of 7	[37]
Chrome tannery effluent treatment using municipal solid waste-derived activated biochar (MSWAB)	The highest removal of 98.97% Cr using 10 mg/mL MSWAB at an optimum pH of 12	[38]
Adsorption of Cr(VI) from wastewater using magnetic-activated carbon	Highest Cr(VI) removal of 99.5% in 100 mg concentration after adsorbent synthesis, pyrolysis, magnetization, and then enhancement with NaOH	[39]

from the ability of the system to withstand high concentration of toxic hexavalent chromium (Cr(VI)) up to 5000 mgL⁻¹ as reported by Wang et al. [20], about 90% and 94% removal of COD and phosphates respectively by Marchetto [27] to the possibility of the treatment system to perform in highly acidic condition like pH of less than 1.0 as reported by Elyahyaoui et al. [34] and Louarrat et al. [26]. Another vital credit associated with these methods is that the high removal efficiency reported was achieved briefly. In other words, it was considered a fast treatment method [40].

Despite these advances in the physio-chemical treatment systems, there is still numerous bottleneck that needs to be addressed for it to be environmental friendly and sustainable wastewater treatment approach the may include the cost of chemicals and energy employed, excessive requirements in terms of mixing and aeration that account for about 45-75% of total treatment cost [41]-[42], incomplete removal

capacity as 20 mgL⁻¹ of Cr(VI) ions concentrations was not able to be removed by precipitation from the maximum concentration of 5000 mgL⁻¹ [20] as well as excessive sludge production, that require further treatment(s) approach.

In addition to that, physio-chemical methods were also connected with single parameter removal, which has been reported to remove only trivalent chromium (Cr(III)) by Wang et al. [20] and Cr(VI) ions removal by Louarrat et al. [26] and Rangasamy et al. [28]. It is also associated with a lack of nutrients assimilation, in which some research reported no prospects. In contrast, others show minimum removal that does not meet with discharge regulation as only 36% of the nutrients were removed by Fettig et al. [17], in which the remaining 64% in the effluent, if not further treated, could affect the lives in aquatic environment by supporting the growth of unwanted plants in the water bodies that are termed as eutrophication.

Biological Treatment Approach

On the other hand, biological treatment methods like the activated sludge process (ASP) were investigated to access the removal of organic and inorganic pollutants with minimal treated effluent discharge risk. One of the critical parameters for these kinds of waste stabilization processes is aeration, which considerably increases the treatment cost. From 1951-1954, Oswald and Gotaas [43] investigated the use of microorganism in sewage treatment of an open pond at laboratory and pilot-scale to establish the relationship of the organisms in waste stabilisation, efficient gas exchange as well as establishing the design criteria for open pond construction and performance of oxidation ponds.

In this process, carbon dioxide (CO₂), water (H₂O), and nutrients from the waste were utilized in the presence of energy from the sun by algae to photosynthesized and grow, and as such, provide more oxygen (O₂) from bacteria in the activated sludge for waste degradation and turn produces more of CO₂ for continuous gas exchange and sewage treatment at a relatively lower cost. Oswald et al. [44] compared the nutrients and gas production ability of raw sewage sludge (A) and algae (B). After volatile organic matter destruction by digestion, the gas production of A is always 2-4 cu ft. higher than that of B. consequently, the nitrogen concentration of B is 7.7% of the total solids as compared to about 3.3% of the total solids of A as digested sludge in this reactor can be dewatered easily while in reactor B, the digested algae were highly gelatinous, colloidal and dewatered poorly, and also contains less degradable substances.

More recently, Haydar et al. [22] evaluated the performance of ASP to treat tannery effluent operated continuously for 267 days at mixed-liquor volatile suspended solids of 3500 mgL⁻¹ for BOD and COD removal. Entirely operated aerobic treatment systems like ASP were employed to explore the capability of microorganisms in waste degradation, such as bacteria, to utilize oxygen available in breaking down the waste substances to more soluble and less toxic compounds. This study involves the use of a 3.15 L aeration tank capacity and 1.1 L settling portion capacity. The removal efficiencies were reported to be 90% and 80%, respectively.

However, despite high removal efficiency associated BOD and COD in ASP, several drawbacks include release of carbon dioxide (CO₂) emissions [45], the excessive land requirement, technicalities and construction cost, big footprint, relatively longer hydraulic retention time (HRT), biomass wash-out [46] and biomass growth rate maintenance that lead to failure of the system. As such, an algal treatment system was also used due to their ability to assimilate nutrients for growth [47], accumulate contaminants [48], adsorbed heavy metal ions [49], [10] and regeneration of adsorbent with adsorbate [50], to treat a variety of wastewater that ranges from domestic, municipal and industrial with varied strength and contamination.

Microalgal Treatment System

Though microalgae have these credits, their cultivation requires the illumination for photosynthesis [4], aeration for effective mass transfer of gasses, nutrients, and growth media [51]. However, these escalate the cost of microalgal production, and hence, a more economical way of algal production is needed to cut the treatment cost. Interestingly, recent studies indicated that microalgae could grow in wastewater [52], thereby assimilating the nutrients for growth. This serves as eutrophication mitigation [53] and saves the cost of nutrients for growth with consequent wastewater treatment.

Aksu et al. [49] investigated the capacity of three dried algal species of *Chlorella Vulgaris*, *Scenedesmus obliquus*, and *Synechocystis* to bioremediate Cu²⁺, Ni²⁺ and Cr(VI) in synthetic tannery wastewater. Specific parameters like pH, initial ion concentration, and biosorbent dose were put under consideration. It was found out that the heavy metal ions removal increases with increasing ions concentrations up to 250 mgL⁻¹. Optimum pH values of 2.0, 4.5, and 5.0 were observed to provide the best removal efficiencies for Cr(VI), Ni²⁺, and Cu²⁺, respectively. The absorption pattern of metal ions was for all the algae species was similar. Among these three species, *Chlorella Vulgaris* has the highest removal capacity with efficiencies of Cu²⁺, Ni²⁺, and Cr(VI) as 38.2%, 42.3%, and 23.0%, respectively, at an initial concentration of 100 mgL⁻¹.

Spirulina fusiformis ability to bioremediate Cr in effluent from the retaining process was investigated by Pandi

et al. [54]. The effluent was diluted to different Cr concentrations of 25, 50, 75, 100, 125, 150, 200, and 300 ppm inoculated in conical flasks under growth conditions of illumination using a white fluorescent lamp 1500 lux, at 25 ± 2 °C for 2 weeks. The result from the study revealed a significant decrease in COD was observed is may be due to the removal of chemical compounds by the organism. At the same time, there is no much BOD reduction as the culture was not heavily loaded with organic substances. The biomass growth was lower than that of the control after 3 weeks due to increased Cr concentration in the growth medium and highest Cr removal efficiencies from the test samples, which ranged between 93-99.5%. The removal efficiencies were higher in Pandi et al. [54] and that result by Aksu et al. [49] due to improvement of the treatment condition of light and growth medium.

Ajayan et al. [55] employed the use tannery wastewater (TWW) to grow *Scenedesmus* sp. and accumulate heavy metals like Cr, Cu, Pb, Zn and nutrients as NO_3^- and PO_4^{3-} . The TWW was diluted with distilled water as 10, 25, 50, 75, and 100%, while the control comprises only BG-11 medium. The initial algal culture concentration for inoculation was 25×10^4 cell/mL, at 150 rpm, for about 27 ± 2 °C in 250 mL Erlenmeyer flask with a light/dark period 8/16 and 150 mL working volume. The highly toxic TWW can support algal growth, and the maximum cell growth was attained in the first eight days as 1180×10^4 cells/mL in a 50% diluted sample. The removal efficiencies of Cr, Cu, Pb, Zn, NO_3^- and PO_4^{3-} were 81.2 – 96, 73.2 – 98, 75 – 98, 65 – 98, >44, and >95%. This higher removal capacity of the treatment may be connected to the treatment conditions of light, temperature as related to the finding of Pandi et al. [54].

Batch biosorption studies using *Spirulina* sp. remove Cr ions from a stock solution of 1000 mgL^{-1} Cr concentration after the dissolution of potassium dichromate by Rezaei [50]. The influence of biomass concentration, adsorbate dose, pH, and agitation time was investigated for the sorption experiment in a 250 mL conical flask. The flasks were agitated at 120 rpm in a temperature-controlled shaker at a predetermined interval of time. Whatman No. 41 filter paper was employed to separate the adsorbate from the adsorbent. The result of the experiment revealed

that the quantity of Cr ions accumulated by *Spirulina* sp. increased with an initial increase in Cr ion concentration and the highest Cr removal efficiency was 82.45% and maximum Cr uptake capacity of 8.25 mg/g with the concentration of an initial ion of 10 mgL^{-1} for 120 min.

In related research, Apandi et al. [56] inoculated *Scenedesmus* sp. in wet market wastewater (WM) in a 500 mL Erlenmeyer flask containing 400 mL wastewater. The wet market wastewater was first filtered through a GF/C filter (Whatman No 1). Microalgal species were first isolated and then cultured in BBM up to 106 cells/mL concentration and then transferred to the WM concentrations of 10, 25, 50, 75, and 100% inoculated for 18 days. The maximum growth rate of *Scenedesmus* sp. was obtained from 50% WM of corresponding dry weight of 2006 mgL^{-1} with a subsequent removal efficiency of Cadmium (Cd), Cr, Fe, and Zn 93.06, 91.50, 92.47, and 92.40%, respectively. Though these findings were related to Ajayan et al. [55], it was more economical in 8 hrs daily illumination.

Subashini & Rajiv [10] investigated the capability of *Chlorella vulgaris* (DPSF 01) as a remediation tool for toxic compounds in tannery effluent. *Chlorella vulgaris* sp. was initially cultured in BBM to the density of 30×10^3 cells/mL, at a temperature range of 20 – 23°C with a 12 hrs light. The *Chlorella vulgaris* was washed using sterile water and then inoculated in tannery effluent of different dilutions with tap water as 0, 25, 40, 55, 70, and 85%. The study results indicated that the maximum growth rate of *Chlorella vulgaris* (DPSF 01) was found to be 760×10^3 cells/mL for 85% dilution solution after 4 weeks. The heavy metals removal of Cu, Zn, Fe, Cr, and Ni was 71, 50, 45, 40, and 20%, respectively.

Even though multiple pollutants removal reported that ranged from heavy metals, organic and inorganic contaminants, with higher remediation capacity up to 99.5% of Cr [54], 98% and 100% of Pb and phosphate [55], and 93.06% of Cd [56], still, low removal efficiencies were reported by Aksu et al. [49] and Subashini & Rajiv [10], with consequent treatment improvement of irradiance supply at 12 photoperiods for 4 weeks and subsequent low BOD, NO_3 and PO_4 removal by Pandi et al. [54]. These might hinder the success of the treatment approach by additional treatment

requirements or incorporating alternative nutrients removal techniques for safe discharge of treated effluent to the waterways. Another bottleneck of algal alone treatment approach is difficulty in microalgal cells settlement for harvesting due to the small size of less than 30 μm [57] and net negative charge of the cell [58].

Hybrid Microalgal Prospects

As mentioned earlier, aerobic treatment is associated with high aeration cost the ranges between 45 – 75% [41]–[42] of the total treatment coupled with excessive sludge production [57] that need further treatment with the consequent escalation of overall cost, but have substantial heterotrophic bacteria with high degradation capability of waste substance and production of CO_2 [59] as a by-product which only required O_2 input. On the other hand, microalgae were said to require CO_2 and assimilate nutrients in the waste stream for growth, and then O_2 was produced [4]. Therefore, a treatment method that can couple the use of these two essential by-products of O_2 and CO_2 could be termed as effective and sustainable since the efficient utilization of these gases reduces the footprint of the biological treatment process, reduces or eliminate aeration cost and may lead to the effective removal of organic and inorganic substances as well as renewable energy alternatives

like biogas, biodiesel and biofuels production from the treated biomass.

Oswald and his colleagues in the 1950s first investigated the use of a microbial community of algae and bacteria to treat sewage. Since then, several attempts were made to improve the efficiency of the system. In line with this, Mohammed et al. [4], Mohammed [45] developed an energy-efficient hybrid mixed-microalgal-activated sludge process as shown in Figure 1 to treat synthetic municipal wastewater in stirred-tank photobioreactors (STPBRs) at an optimum irradiance of $582.7 \mu\text{mol m}^{-2} \text{s}^{-1}$, 12 hrs photoperiod and about 100 rpm stirring rate for 64 days. In the STPBRs, about 95% of energy-saving was reported compared to the conventional activated sludge (AS) process and the highest biomass growth rate and productivity of 0.109 d^{-1} and $0.034 \text{ g L}^{-1} \text{ d}^{-1}$. The maximum highest removal efficiency of soluble chemical oxygen demand and ammonium nitrogen ($\text{NH}_4^+ - \text{N}$) as 70 and 90%, respectively, and this research was limited in heavy metal ions removals.

Tricolici et al. [57] investigated microbial efficiency consortium of *Chlorella Vulgaris* and bacteria in treating dairy wastewater in a 3 L stirred-tank batch bioreactor 96 hrs, 50 rpm at 15/9 hrs light/dark photoperiod illuminated with a white-cool circular lamp at 25,

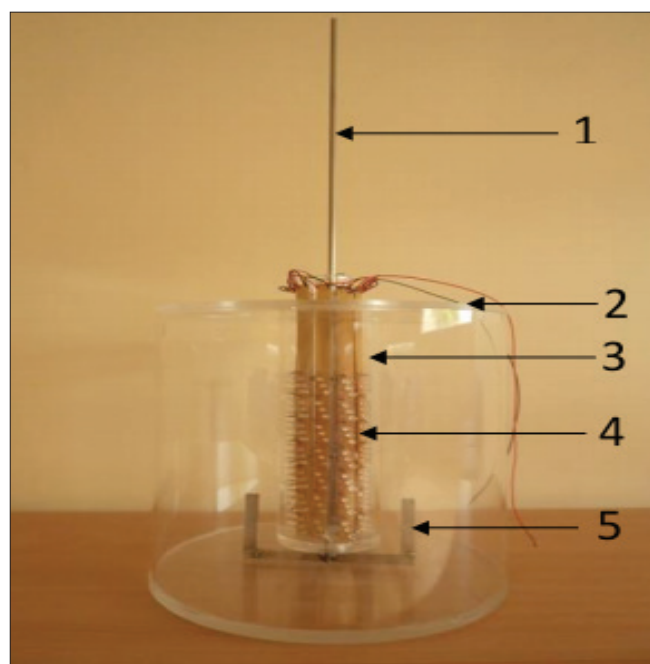
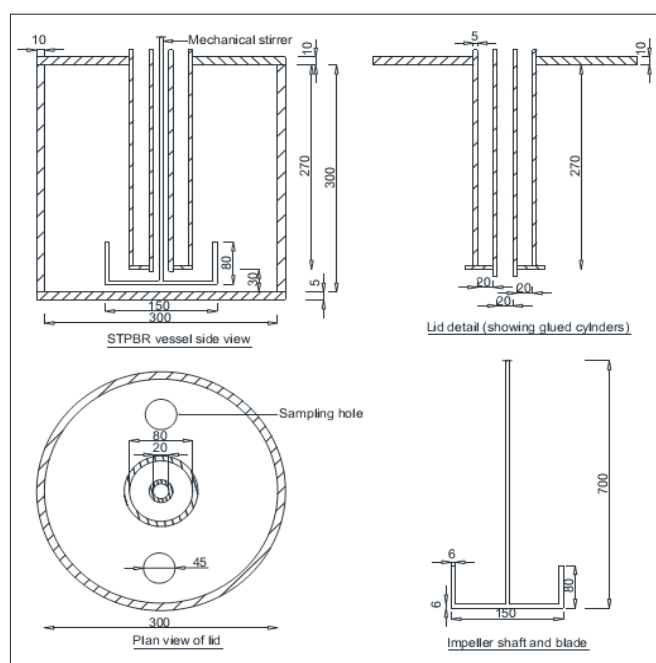


Figure 1 Detailed drawing of the STPBR (all dimensions in mm) and STPBR showing: (1) stirrer shaft, (2) lid holding the LED chamber, (3) LED chamber, (4) LED core, and (5) stirring blade [4],[45]

690 Imm^{-2} . The result shows that the highest removal efficiency of COD, NH_4^+ and PO_4^{3-} were found to be 91, 68, and 38%, respectively, and the maximum growth rates for algal and algal-bacteria system were reported after 72 hrs of batch treatment to be 0.13 and 0.10d^{-1} while problem associated with microalgal harvesting after the treatment was reported.

Apart from gases exchange, Krustok [59] reported a more advanced symbiotic relationship that exists between algal and bacterial cells that ranges between the ability of bacteria to use dead algal cells as nutrients source and bacterial consumption of oxygen produced after photosynthesis help in maintaining the level as its high concentration inhibit photosynthesis and the majority of algal species cannot synthesised their vitamin B_{12} which was produced cheaply by bacteria and promoted aggregation of biomass for settlement and subsequent harvesting. Algae were associated with suppressing biofilm formation, which inhibits bacterial sensing and the capability of microalgae to remove or reduce the pathogenic bacteria that may hinder the growth of other bacterial cells in the treatment systems.

Anbalagan et al. [41] coupled microalgal-activated sludge (MAAS) process for municipal wastewater treatment with subsequent production of renewable energy alternatives in a 22 L semi-continuous photobioreactors R_1 and R_2 with inbuilt agitator for mixing, effective nutrient mass transfer, and to avoid biomass settling. Illuminated with a fluorescent lamp at $150\text{ }\mu\text{molm}^{-2}\text{s}^{-1}$ and operated at 6 days HRT in batch mode with CO_2 addition externally. The result shows more biogas production feasibility of untreated MAAS than the treated, and the maximum total nitrogen removal was about 81 and 64% for six and four days HRT, respectively. These findings support the idea of using MAAS for wastewater treatment, renewable energy production, and reduction of chemical precipitation, aeration, and CO_2 emissions.

Climate summit held in Paris, the United Nations mandated all countries to reduce greenhouse gases (GHGs) emission to reduce the effects of global warming [60], increasing the demand for a sustainable treatment system that may reduce the release of CO_2 emissions. Also, the capabilities of algae to utilize the CO_2 in the presence of light to photosynthesis make them the best

option. Su et al. [60] cultured cyanobacteria species *Thermosynechococcus* in a photobioreactor under high illumination to assess its nutrients assimilation, fixation CO_2 , and potential feedstock for bioethanol production. Under the illumination of $2000\text{ }\mu\text{Em}^{-2}\text{s}^{-1}$ through a 1.5 cm light path in the flat plate photobioreactor, the maximum CO_2 fixation rate, biomass, and glycogen productivities were 221.5, 138.7 and $75.9\text{ mgL}^{-1}\text{h}^{-1}$ and this indicated great potential for greenhouse gases emission reduction with consequent global warming mitigation.

Algal-activated sludge consortia were reported to remove carbon, nitrogen concurrently, and phosphorus in a single treatment operation and, hence, describe the simplicity of the approach and reduce the cost of the operation [61]. The algal strain *Chlorella vulgaris* NIES-227 was cultured using BG-11 media in air-lift photobioreactors under the illumination of $200\text{ }\mu\text{molm}^{-2}\text{s}^{-1}$ continuously with aeration enhancement of about 2% CO_2 by Zhu et al. [61]. Three different batch systems containing (a) the activated sludge only (2.7 gL^{-1} of MLVSS), (b) pure culture of *Chlorella vulgaris* (0.4 gL^{-1}), and (c) the co-culture of *Chlorella vulgaris* and activated sludge at a ratio of 1:1. The maximum NH_4^+-N removal efficiencies of activated sludge, *Chlorella vulgaris*, and co-culture were reported to be 30.0, 77.1, and 75.5%, respectively. The potential of the algal-bacterial consortia treatment approach for biomass production nutrients removal and subsequent wastewater treatment was investigated. However, the information on their removal performance and recoveries was still limited and more needed.

Solé-Bundó et al. [62] employed algal-activated sludge treatment systems to optimize the anaerobic digestion of biomass for valuable renewable energy production components like methane. This treatment system compared the use of both treated and pre-treated microalgae and primary activated sludge for methane production, and 25-75% primary activated sludge concentration was added to the biomass for anaerobic digestion. The highest methane production of $339\text{ mLCH}_4/\text{gVS}$ was obtained when 75% of primary sludge was added to the pre-treated microalgal culture. This was then further investigated in mesophilic lab-scale reactors. Methane production on average for co-digestion to be about $0.46\text{ LCH}_4/\text{gVS}$,

which is 2.9-fold higher than 0.16 LCH₄/gVS obtained from the pre-treated microalgal mono-digestion and it was also indicated that HRT plays a vital role in increasing methane production in which increasing HRT from 20 to 30 days, the methane production increases to about 50%. However, 75% of activated sludge may reduce the light penetration through the culture and affect microalgal growth.

Research on 25/75% of microalgae and activated sludge process conducted by Solé-bundó et al. [63], in which maximum removal efficiency for COD from 72.6 mgL⁻¹ in the influent to 32.9 mgL⁻¹ after treatment and NH₄⁺-N was reduced from 88 mgL⁻¹ to 1 mgL⁻¹ on the contrary Mohammed [45] reported more than 70 and 90% of COD and NH₄⁺-N respectively, using 21L stirred-tank photobioreactors at 582.7 μmolm⁻²s⁻¹ illuminations with red LEDs at 100 rpm when 10% of the activated sludge was mixed with 90% microalgae that also provided maximum specific growth rate and biomass productivity of 0.9 d⁻¹ and 0.034 gL⁻¹d⁻¹ [4] respectively. This lower activated sludge proportion provides an organic substrate for the biodegradation of waste substances. It provides a less dense culture that allows light penetration for the sustained photosynthesis process, and consequent CO₂ and O₂ reported 95% energy savings compared to conventional activated sludge treatment systems [64].

In a related development, Sun et al. [46] obtained activated sludge (AS) from Harbin, China, from the wastewater treatment system to assess the microbial symbiotic relationship in waste treatment. AS was continuously fed into the reactor for acclimation at a TSS concentration of 300 mgL⁻¹, which was used as inoculum in a 2L transparent glass for light transmission and operated as a batch system using 5-set of reactors as R₀, R₁, R₂, R₃, and R₄ with corresponding volatile suspended solids (VSS) of 189, 402, 658, 945 and 1242 mgL⁻¹ respectively. In an AS alone treatment, after 7 days, the maximum removals of soluble chemical oxygen demand (SCOD), ammonium nitrogen (NH₄⁺-N), and phosphate (PO₄³⁻-P) removals were observed in R₂, like 90.6, 97.69, and 83.81%, respectively which was higher than that of R₃ and R₄ due to the moderate activated sludge concentration of 700 mgL⁻¹ in R₂ than 1100 and 1500 mgL⁻¹ in R₃ and R₄. As high sludge concentration in R₄ leads to cell shading of light

among the photosynthetic algal population, increases O₂ consumption due to dark respiration, and all these retard microalgal growth.

Microalgal-activated sludge treatment was a newly emerging technology in pollutants bioremediation with several prospects. However, co-cultures of algae and bacteria were efficient in wastewater treatment but were only tried in the laboratory and pilot-scale reactors sizes. [45],[4],[64] treated municipal wastewater in a range of 1 – 20 L photobioreactors, 15.4 L by Anbalagan et al. [41], 250 mL by Krustok [59], 2 L by Sun et al. [46], 250 mL by Chen et al. [65], 1.5 L by Solé-bundó et al. [63] and more recently 2.5 L reactor by Al-jabri et al. [66]. However, full-scale development of this symbiotic treatment might benefit bioremediation of toxic pollutants, biomass production, and energy and non-energy molecules.

Co-culture of algae and bacteria were employed in the treatment of various wastewater that ranges from treatment of municipal wastewater [4],[41],[64],[65], tannery wastewater [38], high-rate algal pond effluent [63], activated sludge [62] and heavy metal ions removals [67]-[68], with higher removal efficiencies of N-NH₄⁺, N-NO₃⁻, P-PO₄³⁻, COD, BOD, and heavy metals removal efficiencies. However, Mohamed et al. [69] and Mohammed [45] reported a comparatively lower P removal due to the limitation of ammonium nitrogen for polyphosphate accumulation organisms [69]. Further investigations on the performance of microalgal-activated sludge treatment systems on industrial effluent treatment for heavy metals bioremediation is needed, especially with the reported capability of bacterial cells in the biotransformation of toxic metal ions to non-toxic or less toxic substances as in the case of reduction of Cr(VI) to Cr(III) [70]. More recent microalgal and bacterial treatment systems reviews were presented in Table 2.

Future Challenge

Despite the contributions of activated sludge in co-culture of microalgal-activated sludge treatment system in the breaking down of waste substances, utilisation of O₂ produced during photosynthesis, bioaccumulation of the pollutants, biotransformation of toxic pollutants to non-toxic or less toxic, and provision of CO₂ for fixation; little attention was paid to its proportion in this novel treatment approach. Bacterial cells were

Table 2 Recent Reviews on Microalgal-Activated Sludge Process

Treatment	Findings	Reference
Synthetic wastewater was treated with different ratios of algae and activated sludge	The maximum removal of COD, TN, and $P-PO_4^{3-}$ was 82, 65, and 37%, respectively, and the highest removal efficiencies were obtained at a ratio of 1:1 in terms of algal activities and biomass growth	[71]
Synthetic wastewater was treated in a microalgal-activated sludge process	Inadequate P removal due to shortage of polyphosphate accumulating organisms and also no significant variation of results due to varying light intensities	[69]
Wastewater treatment using microalgae	The capability of microalgae to remove N, P, and COD with 87.3% N and 82.9% P removal was reported at 3.1 days hydraulic retention time	[72]
Synthetic wastewater treatment using membrane bioreactors	5.0 mg/L concentration of Cr(VI) significantly influence the growth of <i>Chlorella v.</i> and the Cr(VI) removal decrease with an increasing Cr(VI) dose	[68]
Industrial effluent treatment using microalgal species	The acidic treatment altered the organic ligands on the biomass surface, thereby promoting more electronegative groups and by increasing Al, Ni, and Cu removals	[67]

reported to have a much more growth rate than microalgae; therefore, extensive research is needed to establish an appropriate microbial ratio. To avoid dominance of a species, on the other might lead to system failure. Production of excess O_2 might increase the acidity of the treatment culture, which affects the system's overall performance. In contrast, excess CO_2 production might microbial symbiotic secretions and contribute to greenhouse gas production.

On the other hand, nutrient availability, CO_2 , and light were necessary for microalgal growth and productivity. Therefore the supply of these factors was paramount for sustainable algal growth. Several types of the research reported the ability of microalgae to grow and photosynthesised in wastewater by assimilating nutrients in the waste stream that reduces the cost of artificial fertilisation usage. An efficient proportion of algal-bacterial ratio will guarantee the required quantity of CO_2 production for fixation by microalgae during photosynthesis. Above all, light limitation due to cell shading was associated with a dense culture that prevents the required irradiance from reaching the surface of the microalgal cells as such hinders the photocatalytic reaction and decreases the algal biomass growth and consequent productivities. The algal growth and productivity in microalgal-activated sludge are essential for complete heavy metal and nutrients removals and higher biomass growth for renewable energy production, including biogas, biofuel, biodiesel, and biofertilizers.

Numerous researchers reported different algal-activated sludge ratios as Mohammed [45], Mohammed et al. [4] investigated the influence of 10% activated sludge in the microalgal treatment systems about high removal efficiencies of 70 and 90% for COD and NH_4^+-N , respectively. A 50% activated sludge was studied by Anbalagan et al. [41] and reported a maximum nitrogen removal of 81% at 6 days HRT, and 75% activated sludge concentration was employed by Solé-Bundó et al. [62], Solé-bundó et al. [63] and maximum removal efficiencies of 55 and 88% for COD and NH_4^+-N respectively and 339 mLCH₄/gVS of methane was produced. Also, the highest removal efficiency of SCOD, NH_4^+-N , and $PO_4^{3-}-P$ were observed to be 90.6, 97.69, and 83.81% respectively in R_2 with primary sludge concentration of 700 mgL⁻¹ instead of 1500 mgL⁻¹ in R_4 was reported by Sun et al. [46]. Based on this, a thorough investigation of the ratio from 0 – 100% of microalgae to that of activated sludge in microbial consortia for wastewater treatment is highly required to design an economical, efficient, environmentally friendly, and sustainable treatment approach.

CONCLUSION

A considerable amount of wastewater was produced daily due to human activities that negatively affect both lives and the environment. This wastewater produced was toxic, highly polluted with organic

and inorganic contaminants like heavy metals that are highly carcinogenic and mobile. Several physio-chemical treatment approaches were employed in wastewater treatment to effectively handle high toxic waste constituents with removal efficiencies up to 90%. However, these were associated with the setback of single pollutant removal, incomplete removal, and above all, the cost in terms of chemicals and aeration. The microalgal-activated sludge process was reported to support self-aeration, multiple contaminants removals, and provide renewable energy alternatives. Despite these prospects, there need for thorough investigations on the optimum microalgal-bacteria ratio in the treatment system that will guarantee efficient gases exchange and wastewater treatment and in-depth understanding of the microbial interactions as well as their impacts of their secretions towards the efficient treatment system that will be cost-effective and sustainable treatment systems.

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