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# A Review on Oleaginous Microorganisms for Biological Wastewater Treatment: Current and Future Prospect

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## Abstract

The water scarcity issue is becoming a critical issue to the climate change, industrialization and urbanization. Prompt to the advances in biotechnology, Oleaginous microorganisms have been discovered and successfully applied in biological wastewater treatments, which are highly effective for wastewater clean-up and energy efficient lipid conversion to value-added products. This paper aims to review the recent advances of the application of different types of Oleaginous microorganisms (e.g. yeasts, microalgae, and fungi) as well as the advantages, limitations and application fields (food industry, municipal waste and chemical plant). The future prospect and challenges of Oleaginous microorganism that warrant in environmental settings or engineered systems are also highlighted in the review. In order to improve the Technology Readiness Level (TRL), the future research direction should be more focussed on the economic and environmental studies.

**Keywords**: Oleaginous microorganisms; Biological wastewater treatment; Microalgae; Industrial application

## 1 Introduction

The fresh water demand is rapidly increasing due to urbanization and industrialization, and predicted to beyond than 55% in by the year of 2025 [1]. In recent year, "wastewater treatment field" is becoming a hot topic in both academic and industrial community to eliminate both chemical and microbial pollutants from municipal/industrial wastewater [2]. Nevertheless, the reuse of water from treated wastewater effluents can pose a serios health issue due to contamination such as microbial pollutants, heavy metals, suspended solid and organic matters [3]. To date, biological treatment is well-acknowledged as one of the most eco-friendly and cost-effective way to remove those contaminants from wastewater [1, 4-8]. Notably, the use of Oleaginous microorganisms in biological treatment of wastewater is much attractive as compared with the traditional aerobic digestion and anaerobic digestion technologies which requires high-end system such as up-flow anaerobic sludge blanket digestion or expanded granular sludge bed digestion [9-11].

Apart from cost-effectiveness, Oleaginous microorganisms can clean up the wastewaters effectively within a short period with valuable generation of some value-added products as shown in Fig. 1. For instance, Chlorella pseudolambica has been studied in livestock wastewater for biodiesel production [12]; Sterigmatomyces halophilus has been applied in textile dyeing wastewater for bioremediation [13]; and Chlorella vulgaris has been reported for biochar production using rich in ammonia-N swine wastewater as source of nutrient [14]. Most importantly, the valorisation of Oleaginous microorganisms in low-cost substrates like nutrient-rich wastewaters is a circular economy concept that can help improve the economic feasibility of the wastewater treatment plants related industries with a net positive value. Thus, in this review, we aim to provide a comprehensive insight on the advances of wastewaters treatment by Oleaginous microorganisms which includes 1) The types of Oleaginous microorganisms used for biological treatment such as microalgae, yeast, fungi and bacteria; 2) The industrial application of Oleaginous microorganisms such as food, pharmaceutical and municipal waste industries and also; 3) The commercialization attractiveness and challenges of the technology.

## 2 Types of Oleaginous microorganisms used for biological treatment of wastewaters

Most of the studies have reported that Oleaginous microorganisms accumulate a high lipid content in the range of 14-75% of their dry weight [15-20]. To date, Botryococcus braunii is reported as one of the richest lipid content microorganisms in which 74.5 % of lipid (58.8% nonpolar lipids and 15.7% polar lipids) can be extracted [21].

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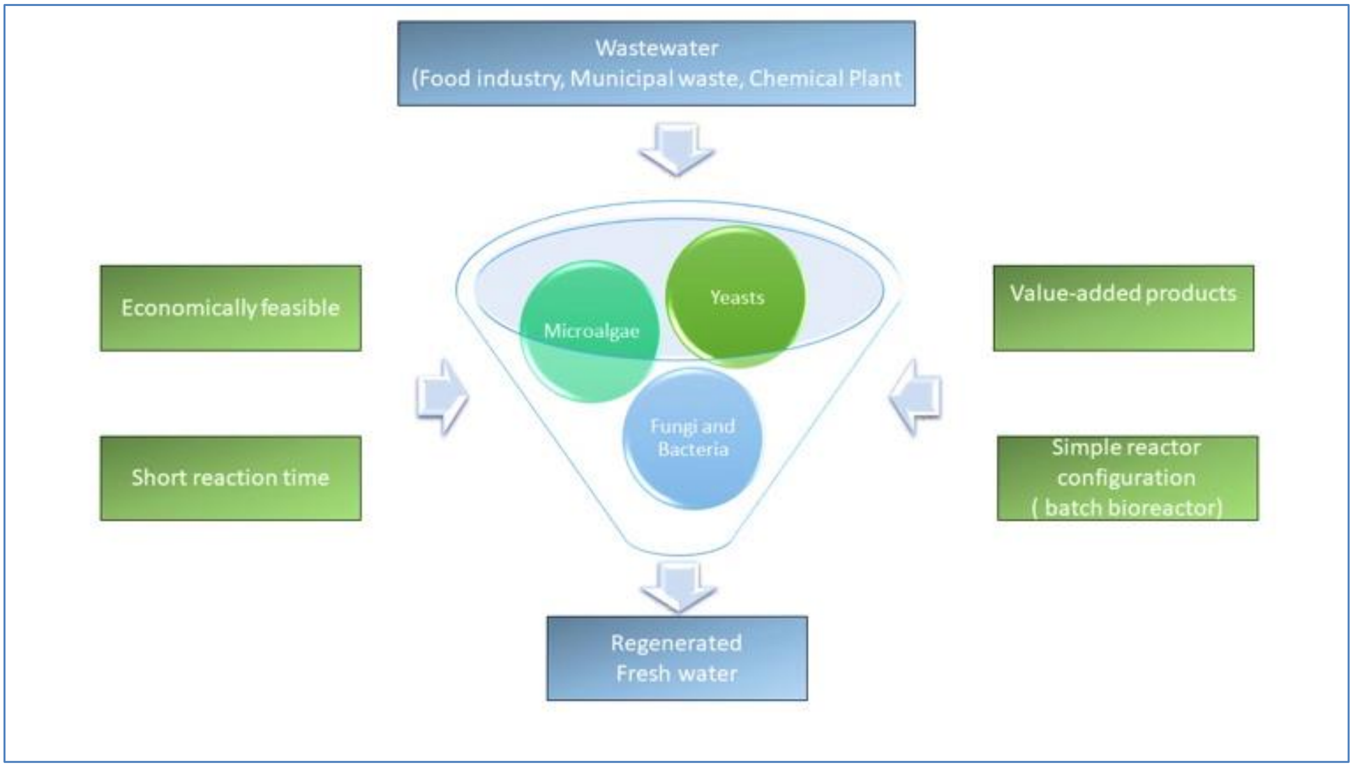


Figure 1: Application of Oleaginous microorganisms for biological treatment of wastewaters

Table 1: Application of Oleaginous microorganisms in biological wastewater treatment for bio-energy production

| Microorganism | Feedstock | Culture mode | Products | Ref |
|---|---|---|---|---|
| *Candida lipolytica* | Molasses | Batch | Biodiesel | [22] |
| *Cryptococcus laurentii* | Winery | Batch | Biodiesel/Bio-oil | [23] |
| *Trichosporon fermentans* | Molasses | Batch | Biodiesel | [24] |
| *Trichosporon dermatis* | Butanol (ABE) fermentation | Batch | Biodiesel | [25] |
| *Chlorella vulgaris* | Artificial wastewater | Batch | Biodiesel | [26] |
| *Scenedesmus sp.* | Starch-containing textile wastewater | Batch | Biogas | [27] |
| *Scenedesmus sp.* | ABE fermentation | Fed-batch | Biogas | [28] |
| *Rhodococcus opacus* | Refinery wastewater | Batch | Bio-oil | [29] |
| *Chlorella vulgaris* | Municipal waste | Batch | Bio-oil | [30] |

Table 2: Application of Oleaginous yeast for wastewaters treatment

| Yeast | Substrate | Biomass (g L-1) | Lipid content (%) | COD removal (%) | Ref |
|---|---|---|---|---|---|
| *Rhodotorula glutinis* | Brewery effluents | 5.2 | 15.0 | - | [36] |
| *Rhodotorula glutinis* | Corn starch farm | 40.0 | 35.0 | 80.0 | [37] |
| *Arthrospira platensis* | Dairy farm | 4.9 | 30.2 | 98.0 | [38] |
| *Rhodococcus opacus* | Dairy farm | 0.7 | 53.2 | 100.0 | [39] |
| *Oleaginous consortium* | Municipal wastewater | 0.6 | 20.0 | 81.0 | [40] |
| *Lipomyces starkeyi* | Potato starch wastewater | 2.6 | 8.9 | - | [41] |
| *Botryococcus braunii* | N-rich wastewater | 2.3 | 30.3 | - | [42] |

Table 1 shows a series of Oleaginious microorganisms that have been utilized for wastewater biological treatment to produce various products such as biodiesel, biogas and bio-oil.

### *2.1 Oleaginous yeasts*

Oleaginous yeasts are capable of accumulating over 20% of their cell mass as intracellular lipids, high growth rate as well as large capacity for substrate consumption [31]. Typical Oleaginous yeasts strains that being investigated are e.g. Yarrowia lipolytica, Rhodotorula glutinis, Rhodosporidium toruloides, Cryptococcus curvatus, Trichosporon pullulan and Lipomyces lipofer as shown in Table 2 [32-34]. For example, Trichosporon cutaneum ACCC 20271 is investigated in an ethanol fermentation wastewater medium by Wang et al. [35]. Without any pre-treatment or external nutrient, it is able to accumulate significant lipid quantities (2.16 g L−1) and remove ca. 55% of COD after a 5-day culture. Meanwhile, Peng's group has reported that Trichosporon dermatis has a high biomass and lipid content of 7.4 g L-1 and 13.5%, respectively [25]. Notably, 68% of COD from butanol fermentation wastewater-based medium is removed after 5 days of fermentation, indicating that Trichosporon dermatis is a potential candidate for large scale wastewater treatment.

### *2.2 Oleaginous Microalgae*

Microalgae are one of the potential candidates for the biological treatment in wastewater facilities, as autotrophic cultivation of microalgae in wastewater open ponds that can enhance the growth as well as the amount of lipid content through consumption of the nutrient the wastewater stream [43-46]. Also, the ambient CO2 can be sequestered for microalgae growth which helps to reduce the global carbon's footprint [47]. Thus, the use of wastewater during the heterotrophic growth conditions for microalgae is an economic feasible process for biodiesel production. The types of Oleaginous microalgae genus reported for simultaneous lipid production and biological wastewater treatment are shown Table 3. In 2011, Feng et al. have replicated an artificial wastewater to cultivate Chlorella vulgaris in a column aeration photobioreactor under batch and semi-continuous configuration. The highest lipids content (42%) with 86% of COD removal and 97% of NH4+ are attained in the semi-continuous cultivation with daily replacement of 1.0 l of the 2.0 l culture [26]. Meanwhile, Hena et al. have also reported similar good result in which that the dairy farm wastewater is a suitable medium for cultivation of Arthrospira Platensis, producing a high biomass yield of 4.98 g L−1 that contains 30.23% of lipids and 98% COD and nutrients. Although the growth period of microalgae is relatively longer when compared with other microorganisms, microalgae is still a better alternative as it can survive in harsh low nutrient concentration wastewaters environment due to its autotrophic character [48].

### *2.3 Oleaginous Fungi and Bacteria*

The application of fungi and bacteria in wastewater purification is much lesser as compared to its counterparts' microalgae and yeasts. Up to date, only a few literature has been reported using Oleaginous fungi namely Aspergillus oryzae [53]¬, Trichoderma reesei ¬[54], Rhodococcus opacus [39], Rhodococcus sp. [55] as shown in Table 4. Minaraj and co-authors have investigated the ability of Rhodococcus opacus in removing the COD of dairy wastewater [53]. They claimed that the bacteria managed to yield up to 14.28% of lipid content and COD removal of 30% without addition of any substrate. Meanwhile, with the addition of mineral salts as external substrate, the lipid content and the COD removal efficiency have increases up to 33% and 62%, respectively. Similar observation has been obtained by Gupta et al where the application of Rhodococcus opacus could remove up to almost 100% of COD with a maximum lipid yield of 1.8 g L−1 at a retention time of 6.6 hr [39].

## 3 Application of Wastewater Treatment by Oleaginous Microorganisms

### *3.1 Food industry*

The strategy of using Oleaginous microorganism for treating wastewater from food waste industry are gaining popularity in solving wastewater pollution and reducing the greenhouse emission [53, 56, 57]. Recently, many researchers have demonstrated that Oleaginous microorganism can be used to treat wastewaters discharged from a wide spectrum of food industries including Monosodium glutamate [58], olive oil [59], soybean [60], and starch [61]. The rationale behind such application is due to wastewater from food industry has high BOD and COD contents, which contributes to its intrinsically high fermentability. Thus, it can be treated easily from conventional anaerobic digestion method. Anbarasan et al. have inoculated Metschnikowia Pulcherrima using the distillery wastewater and produced biodiesel from the lipids accumulated by the microorganism [62].

Table 3: Application of Oleaginous microalgae for wastewaters treatment

| **Microalgae** | **Substrate** | **Biomass (g L-1)** | **Lipid content (%)** | **COD removal (%)** | **Ref** |
|---|---|---|---|---|---|
| *Chlorella protothecoides* | Thiocyanate wastewater | 1.3 | 30.6 | - | [49] |
| *Arthrospira platensis* | Olive-oil mill wastewater | 1.7 | 16.9 | 73.1 | [50] |
| *Aspergillus sp.* | Corncob waste liquor | 2.0 | 22.1 | 60.0 | [51] |
| *Botryococcus braunii* | N-rich wastewater | 2.2 | 30.2 | - | [42] |
| *Scenedesmus obliquus* | Secondary effluent | - | 17.0 | 1.3 | [52] |
| *Scenedesmus sp.* | Secondary effluent | - | 12.7 | 1.0 | [52] |
| *Scenedesmus quadricauda* | Secondary effluent | - | 66.1 | 1.7 | [52] |

Table 4: Application of Oleaginous fungi and bacteria for wastewaters treatment

| **Fungi and Bacteria** | **Substrate** | **Biomass (g L-1)** | **Lipid content (%)** | **COD removal (%)** | **Ref** |
|---|---|---|---|---|---|
| Aspergillus oryzae | Potato processing wastewater | - | 3.5 g/L | 91.0 | [53] |
| Mucor circinelloides | Equalization tank wastewater | 0.6 | 22.1 | 88.7 | [54] |
| Trichoderma reesei | Equalization tank wastewater | 0.7 | 9.8 | 86.7 | [54] |
| Rhodococcus opacus | Dairy wastewater | - | 53.2 | 100 | [39] |
| Rhodococcus opacus | Primary effluent | - | - | 81.0 | [40] |
| Rhodococcus sp. RHA1 | Thermomechanical pulping effluent | - | - | 48.0 | [55] |

The in-situ transesterification reaction is performed using sodium hydroxide and methanol under base catalysis and the biodiesel yield was reported as high as 1.4 g/L. Furthermore, Xue et al. have demonstrated an excellent COD removal of 80 % by treating potato processing wastewater using Rhodotorula glutinis[63]. The COD removal performance in former study is comparably higher than that reported in previous studies of Honyang et.al [64] and Muniraj et.al [65]. In a recent study, a mix-culture of yeast (Rhodosporidum. Toruloides) and microalgae (Chlorella vulgaris) is introduced in a culturing and treating the food waste hydrolysate (see Fig. 2(a)) [66]. Based on the findings, the mix-culture of yeast and culture promoted a higher removal performance of organic matters from wastewater and better lipid production at a shorter cultivation time as compared to the pure culture of yeast and microalgae. Under the mutualistic relationship of both yeast and microalgae, yeasts provide CO2 for microalgae meanwhile microalgae offer oxygen for the yeasts. Furthermore, yeasts mainly consume organic matters and microalgae uptake nitrogen and phosphorus from the wastewater. Thus, mix-culture of yeast and microalgae using food waste hydrolysate as a culture medium is a dual propose strategy in solving the waste disposal issues and alarming energy crisis. As evidence in Fig. 2(b), it showed that the final biomass and substrate utilization ratio of mixed culture is highest at 20% inoculum size of Chlorella vulgaris, the lipid production, lipid content, biomass yield and lipid yield are highest at 10% when the Rhodosporidum. Toruloides Inoculum size fixed at 5%. However, the application of yeast is generally less effective in nutrient removal due to its poor resistance to high organic matter non-sterile wastewater. Up to now, only a few studies have been performed to produce microbial lipid from high strength non-sterile wastewater in the absence of other nutrients for the mixed culture of yeast and microalgae. Future research works should be conducted on producing microbial lipid from non-sterile wastewater as the results could give an insight on bridging the gap between research and industrial practice.

### *3.2 Municipal wastewater*

Lately, there has been a great upsurge of interest in studies related to several aspect of municipal wastewater treatment. Amongst the treatment methods reported in literature, anaerobic-aerobic digestion treatment is one of the methods widely reported and used in treating the municipal wastewater [67-69]. In recent years, Oleaginous microorganism has been proven as one of the promising biological sources in treating municipal wastewater. For example, Goswami et al. explored on the valorisation of biomass gasification wastewater for lipid accumulation by using Rhodococcus apacus and the potential application of biodiesel production [70]. Using the raw biomass gasification water as the synthetic mineral media, the high cell density lipid rich bacterium exhibited an excellent lipid yield of 54.3% with high wastewater COD removal efficiency of 64%.

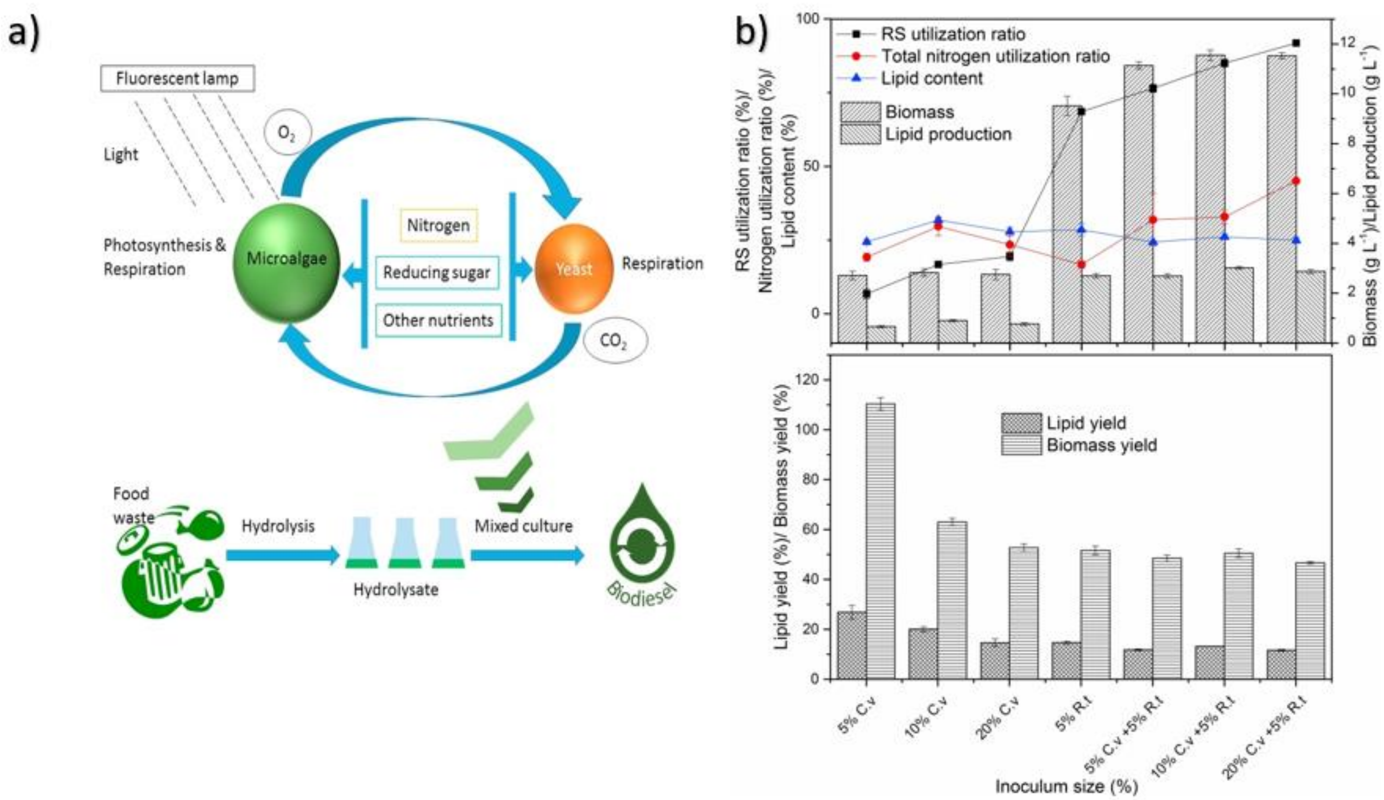


Figure 2: (a) Application of co-culture of microalgae and yeast in treating wastewater derived from food industry, (b) Nitrogen utilization ratio and lipid content extraction from Pure and mixed culture of R. toruloides and C. vulgaris at different inoculum size ratio in FWH. (adapted from [62])

From the transesterification of bacterial lipids to biodiesel, it is revealed that the Rhodococcus apacus bacterial strain has a good potential in treating the biomass gasification wastewater and producing biodiesel. In the same vein, Eida et al. have isolated a local Scenedesmus obliquus from wastewater swamp and cultivated it using secondary treated domestic wastewater for biomass and lipid production [71]. From the results, the secondary treated municipal wastewater is proven to be an economical growth medium for the microalgae cultivation and lipid production. Similar to treatment of food industry wastewater, co-culture of Oleaginous yeast and algae is also reported to be one of the effective strategies in treating municipal wastewater and producing lipids.

In another study of Cho et.al [72], instead of using a single strain of microalgae, a consortium of indigenous microalgae and bacterial from raw municipal wastewater is used for biomass and lipid production. Three different cultivation phases of microalgae and bacteria in raw municipal wastewater in lab-scale photobioreactors operated in circulating batch mode are investigated. From Fig.3, the initial phase I cultivation is performed using only raw municipal wastewater, where phase II and III are replaced with effluent from the sewage sludge fermentation. As a result, a stepwise increment of biologically produced volatile fatty acids in phase II and III can be observed at the beginning of phase II and III. The highest algal biomass production of 117.1 + 2.7 mg/L/d and highest lipid productivity of 17.2 + 0.2 mg/L/d are attained at phase II and III, respectively. As a whole, the stepwise additional of biologically produced volatile fatty acids promoted the microalgae biomass and lipid productions with better nutrient removal performance. However, future studies must be performed to isolate the consortium of indigenous microalgae and bacterial from raw municipal wastewater and expound the individual effect of each microalgae or bacterial strain to biomass and lipid productivity in a photobioreactor. Likewise, consortium of Oleaginous yeasts and bacterium also exhibited excellent COD removal performance (above 81%) when it is used to treat municipal wastewater [73, 74].

### *3.3 Chemical plant*

Wastewater pollution from chemical plants is widely recognized as one of the serious threats to human population and ecosystem due to the discharge of toxic effluents to the surrounding environments. To combat this challenge, the application of Oleaginous bacterial, yeast and microalgae in an integrated application of toxic chemical removal and sustainable biodiesel production have been studied and reported in many previous studies. For instance, pulp and paper industry is one of the main environmental polluters after oil, leather, cement, steel and textile industries. According to the literature, approximately 100 million kg of hazardous contaminants including sodium hydroxide, chlorinated phenol, lignin and other derivatives with high COD are discharged into the environment every year from the paper industry [75]. This raised serious environmental concerns and consequences to our wildlands and communities.

Fig. 4 illustrates the process flow of pulp and paper industry wastewater for Oleaginious yeast cultivation and biodiesel production. It is reported that Rhodosporidium kratochvilovae has unique ability to utilize the pulp and paper industry effluent as a culture medium and accumulate high quantity of triacylglycerol or neutral lipids (8.56 g/L).

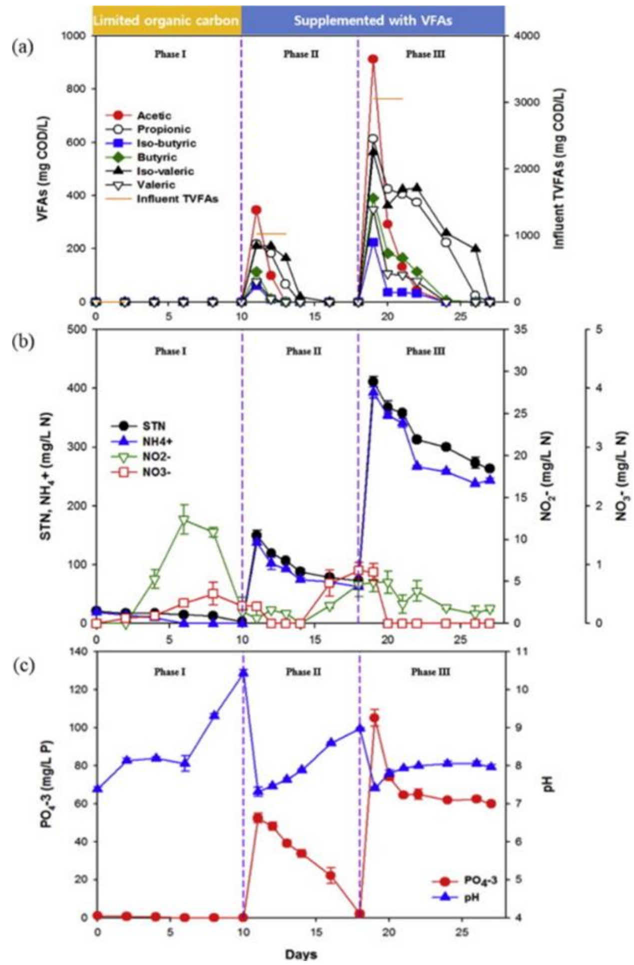


Figure 3: Changes of chemical components in consortium of microalgae and bacterial with stepwise increment of biologically produced volatile fatty acids circulating within the photobioreactor: (a) VFAs, (b) STN, NH4+, NO2−, and NO3−, (c) pH and PO4−3. Ad Adopted from [68]

It also possessed superior phenol (99.60%) and lignin (94.2%) removal performances with high COD (94.22%) and BOD (84.59%) removal efficiencies [75]. Interestingly, the biodiesel obtained from the extracted triacylglycerol has desired biofuel properties including high cetane number, better oxidation stability, and improved cold flow properties. Taking Deeba et al. study as an example, the Cryptococcus vishniaccii (MTCC 232) strain has been used to convert paper mill sludge into neutral lipids for sustainable biodiesel production. From the results, the paper mill sludge extracts have all the essential nutrients for the culture of Oleaginious yeast and the bacterial strain exhibited enhanced triaclyglycerides production of 5.5 + 0.8 g/L [76]. Also, the biodiesel obtained from the transesterification of the accumulated triglycerides is enriched in oleic acid, palmitic acid, linoleic acid, and stearic acid with better oxidative stability and biodiesel quality.

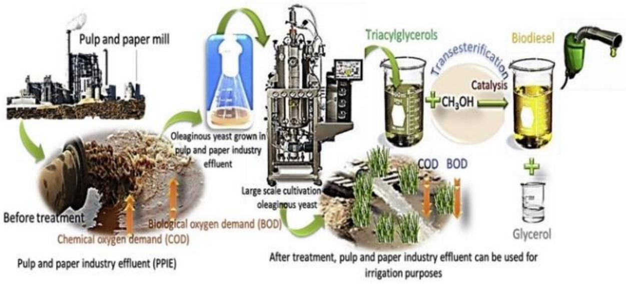

Figure 4: Utilization of pulp and paper industry effluent in cultivating Oleaginous yeast and produc*ing sustainable biodiesel [71]*

Other than paper and pulp industry, industrialization processes including textile, leather, dyeing, cosmetic and pharmaceutical industries have an increasing demand for synthetic lignin-like dyes. Discharge of such hazardous effluents from these industries into water bodies will ultimately give rise to detrimental effects to the environments and aquatic flora. Besides that, the use of oleaginous microorganism including yeast, bacterial and fungi offers another environmentally friendly biological remediation method to decolorize recalcitrant synthetic dyes and valorising lignin while producing sustainable biodiesel. Such method also offers low operating cost, low energy requirement, easy process control, and also the excellent operation flexibility under a wide range of conditions.

In a recent study of Ali et.a l [77], a novel oleaginous yeast consortium, OYC-YBC.SH is developed using three yeast cultures (Viz. Yarrowia sp. SSA1642, Barnettozyma californica SSA1518 and Sterigmatomyces halophilus SSA1511) for textile dye removal, lignin valorisation and lipid production. The oleaginous yeast consortium exhibited superior decolorization performance when tested with real dyeing effluent sample at pH 8. It is able to grow on a wide range of carbon sources with the highest lipid productivity of 1.56 g/L/day and lipid activity of 170.3 U/mL Moreover, substantial detoxification performance is also observed when the textile effluent sample is treated with OYC-Y.BC.SH consortium under static conditions at 30 °C for 24 hrs, which further suggesting its suitability for degrading most of the textile constituents under alkaline pH environments. The TOC, BOD, COD and color removal performances are reported as 54%, 74%, 95% and 98%, respectively.

## 4 Challenges of the application of oleaginous microorganisms in industrial wastewater treatment

As discussed in the above, the use of oleaginous microorganisms is a breakthrough in wastewater treatment as they are inexpensive, environmentally friendly, simple system configuration as well as short fermentation period [78-80]. However, the COD removal by oleaginous microorganisms is still far lower than that of traditional chemical technologies. In fact, the total COD removal of industrial and municipal wastewaters can be up to 95% by using the ion exchange or chemical reduction [81-83]. On top of that, the COD removal obtained by the conventional biological anaerobic–aerobic biological treatment (80-90%) is also much higher than the use of oleaginous microorganisms [67].

## 2 Conclusions

The application of Oleaginous microorganisms is a win-win strategy where the nutrient-rich wastewaters can be act as the medium to produce bioenergy and also, the unwanted nutrient or COD can be removed simultaneously. Owing to its advantages, the application of oleaginous microorganisms in industrial scale seems feasible only if the challenges mentioned above can be resolved. The future research direction should be more focussed on the economic and environmental studies in order to benchmark with the conventional biological (anaerobic) and chemical wastewater treatment.

## Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

## Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

## Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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