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Review

# 미세조류와 박테리아의 공생 배양을 이용한 하폐수 고도처리

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# Advanced Treatment of Wastewater Using Symbiotic Co-culture of Microalgae and Bacteria

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초 록

미세조류와 박테리아의 공배양 시스템은 두 미생물종이 공생적 관계가 있다면 한 배양기에서 BOD와 영양염류의 동시 제거가 가능하다. 이때 영양염류는 미세조류의 바이오매스 성분으로 전환된다. 이 총설은 미세조류와 박테리아의 공생적 혼합배양을 이용한 하폐수처리, 특히 질소와 인의 제거에서의 중요성과 최근의 연구동향을 살펴보았다. 미세조류는 광합성을 통해 산소를 발생시키고 박테리아는 이 산소를 전자수용체로 이용하여 유기물의 산화분해에 활용할 수 있다. 호기성 박테리아가 유기물을 산화할 때 발생되는 $CO_2$는 미세조류의 탄소원으로 섭취되어 탄소동화작용에 사용된다. 미세조류와 박테리아의 공배양은 상호 이익이 될 수도 있고 저해가 될 수도 있으므로 지속적인 영양염류 제거를 위해서는 상호 이익이 되는 공생적 관계가 필수적으로 요구된다. 이를 위해서는 하폐수처리에 사용되는 상용적인 두 미생물 종의 선택이 중요하다.

**Abstract**

The co-culture system of microalgae and bacteria enables simultaneous removal of BOD and nutrients in a single reactor if the pair of microorganisms is symbiotic. In this case, nutrients are converted to biomass constituents of microalgae. This review highlights the importance and recent researches using symbiotic co-culture system of microalgae and bacteria in wastewater treatment, focusing on the removal of nitrogen and phosphorus. During wastewater treatment, the microalgae produces molecular oxygen through photosynthesis, which can be used as an electron acceptor by aerobic bacteria to degrade organic pollutants. The released $CO_2$ during the bacterial mineralization can then be consumed by microalgae as a carbon source in photosynthesis. Microalgae and bacteria in the co-culture system could cooperate or compete each other for resources. In the context of wastewater treatment, positive relationships are prerequisite to accomplish the sustainable removal of nutrients. Therefore, the selection of compatible species is very important if the co-culture has to be utilized in wastewater treatment.

***Keywords:*** *Microalgae, bacteria, symbiotic co-culture, nutrients removal, wastewater treatment*

## 1. Introduction

The excess of nitrogen and phosphorus in surface and marine waters is a common environmental problem around the world. It brings about an increase in algae and aquatic plants, loss of species diversity, and loss of ecosystem function in water body[1]. It can disturb the balance of the ecosystem and as a result, threaten the safety of drinking water[2]. Therefore, these nutrients (N and P) should be removed from wastewater before they discharge into an aquatic environment.

Typical wastewater treatment plants, generally equipped with secondary treatment processes for removal of biological oxygen demand (BOD), are unable to achieve sufficient removal of nutrients. Thus, the tertiary treatment process (or advanced treatment) is now essential in treatment plants for adequate removal of nitrogen and phosphorus. To achieve this goal, biological nutrient removal systems (BNRs) have been extensively used in past several decades; for example, Bardenpho, sequencing batch reactor, and anaerobic-anoxic-oxic ($A^2O$) method, and their modifications[3]. Among them, $A^2O$ process is a fundamental and the most commonly used process that includes anaerobic, anoxic, and aerobic phases in sequence for wastewater treatment. Subsequently, the removal of nitrogen, phosphorous, and BOD would be accomplished in separate phases of the system: nitrogen in aerobic and anoxic through nitrification and denitrification, respectively; phosphorus in anaerobic and anoxic; and BOD in aerobic. In this system, denitrifiers are mainly responsible for nitrogen removal while phosphate-accumu-

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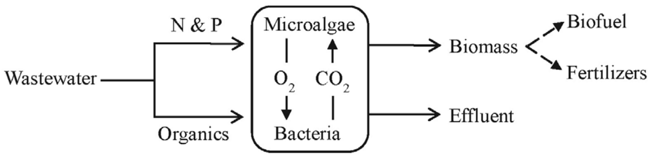



**Figure 1. Schematic diagram of algal-bacterial symbiotic interactions in wastewater treatment.**

lating organisms are responsible for enhanced biological phosphorus removal[3]. Also most BNR processes contain complex treatment steps and are very energy intensive, representing 60-80% of total energy requirement for wastewater treatment[4]. Therefore, the single-step treatment process is needed to achieve simple and cost-effective removal of nutrients from wastewater.

Microalgae have gained huge research interests due to their variety of applications in different sectors, as in biofuel production, wastewater treatment, greenhouse gas abatement, and nutrition and pharmaceutical industries[5]. Microalgae provide an attractive solution for advanced treatment because they have a potential to assimilate nitrogen and phosphorus in their growth cycle. In wastewater, nutrients are mostly present in forms of ammonium, nitrates, and orthophosphates. Microalgae can convert inorganic nitrogen and phosphorus to their organic forms through assimilation and phosphorylation processes, respectively[6]. The Major benefits of using microalgae for tertiary treatment are (i) less cost of the process, (ii) possibility of recycling assimilated nitrogen and phosphorus as fertilizer, and (iii) release of oxygenated effluent into the water body[1]. Hence, utilizing microalgae is an environmentally friendly way to treat wastewater, and successful use of microalgae has confirmed in removing pollutants from fecal, domestic, and industrial wastewaters. Among other microalgal species, *Chlorella* sp. has a vast potential in wastewater treatment because it can tolerate severity of wastewater, uptake nutrients efficiently, and exhibit fast growth rate and short generation time[7]. Ruiz-Marin et al. reported that *Chlorella vulgaris* have shown nitrogen and phosphorus removal performances of 55-88% and 12-100%, respectively from municipal wastewater[8].

Removal performance of nutrients by microalgae in the presence of bacteria can be enhanced. And, by culturing microalgae and bacteria together (as a co-culture system), an efficient and simultaneous removal of BOD and nutrients may be achieved in a single reactor (since multiple reactors are required in conventional BNRs), if growth and maintenance of both microorganisms are compatible. Microalgae have a good potential to assimilate nitrogen and phosphorus; bacteria, on the other hand, can break down organic matters present in wastewater[9]. Effect of symbiotic co-culture involves photosynthetic oxygen production which is utilized in bacterial respiration to oxidize organic compounds and, as a result, inorganic carbon such as $CO_2$ (an end product of bacterial oxidation) is consumed by microalgae as a carbon source[10]. Figure 1 shows symbiotic relationship between microalgae and bacteria during wastewater treatment. The co-culture system for the removal of nutrients and BOD has been considered as an alternative biosystem for wastewater treatment.

Substantial amount of research has been done on the interaction between microalgae and bacteria in wastewater treatment since bacteria naturally exist in wastewaters. Mutually-beneficent interaction is vital for an effective wastewater treatment by the co-culture. Microalgae and bacteria in the co-culture system may encourage mutual growth by releasing growth promoters (mostly organic matters) in the culture medium, and consequently increases removal performance for nutrients[11]. For instance, the growth of *C. vulgaris* was increased when co-cultured with bacterium *Azospirillum brasilense*[12]. And, the efficiency of nutrients removal by microalgae could be enhanced in the co-culture system as compared to the single algal system[13]. BOD removal efficiency of bacteria was also enhanced in co-culture system[14]. This biosystem is ideal as nutrients can be recovered from wastewater as biomass constituents. And, the biomass harvested from effluent can be used as a sustainable substrate (or feedstock) for biofuels or commercial products like drugs and fertilizer[15].

This paper highlights the importance of using the co-culture system of microalgae and bacteria in wastewater treatment. Recent researches on the co-culture systems and their applications were introduced, and the inhibitory/stimulatory effects of microalgae and bacteria in the co-culture system were discussed. Then, the applications of symbiotic co-culture were addressed in detail for the purpose of nutrients removal with comparisons of several consortiums in removing nitrogen and phosphorus from different sources of wastewaters.

## 2. Interactions between Microalgae and Bacteria

In natural environments, microalgae and bacteria exist together. As a result, they have shown beneficial or harmful relationships. Bacteria can promote or inhibit algal growth by producing growth factors or phycotoxins, respectively. On the other hand, algae can also inhibit or promote bacterial growth by producing exotoxins or growth factors, respectively[16]. Interactions between microalgae and bacteria vary from species to species; and depend on environmental conditions[17]. Microalgae and bacteria in the co-culture system can cooperate with each other or compete for resources[18]. For example, in a nutrients-limited environment (such as phosphate limitation), the competition for phosphate would suppress interaction between microalgae and bacteria[19]. Liang et al. found that bacterial growth inhibited in the presence of *C. vulgaris* because of resource competition[20]. *Pseudomonas*, *Flavobacterium*, and *Xanthomonas* (which were in association with *Oscillatoria* sp.) either inhibited or promoted growth of algal species[21]. Thus, microalgae-bacteria interactions range from symbiotic to parasitic. However, in the context of wastewater treatment, positive relationships are prerequisite to accomplish sustainable removal of nutrients. Therefore, an appropriate selection of relevant species is needed in utilizing co-culture system for nutrients removal from wastewater.

Symbiotic relationships (positive/negative) between microalgae and bacteria can be useful for various purposes. For example, the growth-inhibiting effect of bacteria on algae can be used as a biological method to control harmful algal blooms in water bodies[22].

Microalgae and their associated bacteria can be used as a source of artificial food (or food additives) to cultivate aquatic organisms based on their positive trophic interactions[20]. Attachment of bacteria to algal cell surfaces facilitates flocculation and subsequently increases sedimentation rates[23]. Bacteria and their extracellular polymeric substances have a role in enhancing the flocculating activity of algae. For instance, three microalgae-associated bacteria (i.e., *Flavobacterium*, *Terrimonas* and *Sphingobacterium*) and their metabolites had combined role for the harvesting of *C. vulgaris*. These bacteria played a crucial role in bigger floc formation resulting in settleable flocs, which eventually encourages harvesting of microalgae[24]. There are many ways through which microalgae can be harmful or beneficial to bacteria, and vice versa.

### 2.1. Harmful interactions

Microalgae can inhibit activities of bacteria by increasing pH, dissolved oxygen, and temperature of culture medium[25]. Microalgae could also reduce the growth of bacteria by releasing antibacterial metabolites[26]. For instance, bacterial growth repressed by microalgae due to the production of chlorellin (an antibacterial substance)[27]. These effects of microalgae are critical in a situation where monoculture or axenic culture has to grow. In the treatment of wastewater, the maintenance of bacteria is also important since they are mainly responsible for BOD removal. Therefore, by choosing compatible microbial species, harmful effects of microalgae should be avoided. The control of an appropriate culture condition is also important because there should be favorable environmental conditions for both of microorganisms in the co-culturing system.

On the other hand, bacteria can inhibit microalgal growth too by secreting harmful chemicals, such as algicidal extracellular metabolites[28]. It is known that some bacteria release enzymes that can degrade algal cell wall[29]. Cell lysis of algae is noted due to the excretion of bacterial extracellular substances[30]. Bacteria can decrease algal growth by altering their stoichiometry; there was a different chemical composition of microalgae in the co-culture system[31]. The senescence of *C. vulgaris* was observed by the natural association with bacterium *Phyllobacterium myrsinaceous*[32]. A bacterial strain of *Flavobacterium* sp. showed harmful effects on growth of microalgae *Gymnodinium mikimotoi*[28]. Bacterial infection can affect both growth and nutrients removal performance of microalgae[33]. These harmful effects of bacteria are species-specific. In some cases, negative associations are important to achieve a desired outcome. However, in wastewater treatment process, positive interactions are more important.

### 2.2. Beneficial interactions

Microalgae can increase bacterial activity by releasing extracellular substances such as proteins, lipids, and nucleic acids etc.[9]. These substances (which releases in algal growth process) serve as substrates for bacterial growth. Microalgae can provide nutrients for bacterial growth from decomposed algal cells[29,34]. Microalgae can also provide nutrients to bacteria for the synthesis of essential products like vitamin $B_{12}$[35]. Algae can protect bacteria from unfavorable environments by providing them secondary habitat[34]. The growth of *Escherichia coli* improved due to the release of compounds by *Chlorella* sp.[36]. Wolfaardt et al.[37] reported that pollutant removal performance of bacteria enhanced by algal metabolites.

Bacteria can play a beneficial role too in algal metabolism by secreting growth promoters (chemical substances). A significant promotion in growth of microalgae has reported due to vitamin and phytohormones produced by bacteria[12,35]. It was found that the algal growth has promoted because of indole-3-acetic acid (a phytohormone) which was produced by bacteria[38]. Bacteria can help microalgae by decreasing oxygen tension from the culture[39]. Bacteria can convert persistent compounds to nutrients (N and P) and $CO_2$ that can easily use in algal photosynthesis[40]. Microalgae gain energy and nutrients when bacteria release extracellular enzymes that can break down large molecules into smaller ones and also that can degrade organic matters to carbonate, nitrate, phosphate, and sulfate[41].

Efficient exchange of nutrients is possible in the co-culture system because bacterial attachment to the microalgal surface can improve mass transfer by reducing diffusion distance[17]. For example, bacteria *Halomonas* provided vitamin cobalamin to microalga *Amphidinium operculatum*[35]. There was a promotion in algal growth when *C. vulgaris* was cultured together with bacterium *Bacillus pumilus* that fixes nitrogen from the atmosphere for microalgae[42]. In the study of de-Bashan et al.[43], bacterium *Azospirillum brasilense* enhanced nutrients uptake capability of *C. vulgaris*.

## 3. Growth Enhancement of Microalgae by Bacteria

The cooperative interaction between microalgae and bacteria is considered as a strategy to enhance microalgal biomass production. If bacteria could promote the growth of microalgae, subsequently, the performance of nutrients removal by microalgae would be enhanced. Therefore, the selection of well-suited members in the co-culture system is an essential step for sustainable nutrients removal process.

There are several reports on the stimulating effect of bacteria on algal growth. For example, the growth of alga *Asterionella glacialis* could be enhanced because of glycoprotein production by *Pseudomonas* sp.[44]. Two species of *Pseudomonas* (*P. diminuate* and *P. vesicularis*) were capable of increasing the growth of *Chlorella* sp. and *Scenedesmus bicellularis* by creating more favorable environmental conditions, such as the reduction of photosynthetic oxygen tension[39]. The existence of *Pseudomonas* resulted in about 1.4 times higher cell concentration of *C. vulgaris* during a given period than that in single algal culture[5]. Du et al.[45] have confirmed that the decomposing activities of bacteria can enhance the growth rate of microalgae. There was an enhancement in the growth of *Microcystis aeruginosa* due to bacterial decomposition of dissolved organic nitrogen. The growth of marine diatom *Chaetoceros gracilis* in the presence of bacterial strain could be improved[46]. Higher growth of *Chlorella ellipsoidea* has achieved by co-inoculation with *Brevundimonas* sp.[17].

Gonzalez and Bashan[12] reported an enhancement in the growth of *C. vulgaris* due to the synthesis of phytohormones by *Azospirillum* *brasilense*. *A. brasilense* significantly enhanced all growth parameters (such as general population, colony size, biomass, and in some strains, cell size) of *C. vulgaris* and *Chlorella sorokiniana* when co-immobilized with microalgae in small alginate beads. Moreover, many cytological, physiological, and biochemical pathways and metabolites within microalgal cells, including photosynthetic pigments, lipid content, and variety of fatty acids, were significantly changed in co-immobilized system[47]. Similarly, by co-culturing cyanobacterium *Synechocystis* with *Pseudomonas sp.* in biofilm mat, there was an increase in cyanobacterial biomass[48]. It indicates that the biomass of algae can even increase, due to the bacterial stimulation effects, in both suspended and immobilized cultures.

These studies showed the enhancement effects of bacteria on algal growth either by creating more desirable condition for microalgae or by providing some growth promoting substances. The significance of co-culture systems is not just limited to suspended growth reactors. Therefore, positive associations between microalgae and bacteria would be very useful in wastewater treatment especially for the removal of nitrogen and phosphorus.

## 4. Nutrients Removal by Symbiotic Co-Culture

Mutually symbiotic microalgal-bacterial relationship has been used in the wastewater treatment. Single microorganism (microalgae or bacteria) may not accomplish certain tasks more effectively than in co-culture. Co-culture systems can also replace those systems which otherwise require multiple steps to complete a function. The advantages of using co-culture include: strength against environmental instabilities, permanence for the partners, sharing of metabolites and nutrient limitations, and control against invading species[18]. Co-culture can be cultivated in open ponds or closed photobioreactors. Open pond systems are more promising than photobioreactors from an energetically point of view (For an instance, 1 W/$m^3$ for open ponds while 50-300 W/$m^3$ for photobioreactors)[49]. However, some disadvantages, such as evaporative losses and risk of contamination etc., are also involved in using open ponds. Such problems can overcome in utilizing closed photobioreactors where elaborated control of culture conditions is possible.

### 4.1. Algal-bacterial symbiosis during wastewater treatment

In general, microalgae produce molecular oxygen through photosynthesis which is used as an electron acceptor by aerobic bacteria to degrade organic pollutants. The $CO_2$ released during the bacterial mineralization can be consumed by microalgae as a carbon source for photosynthesis[18,50]. In this way, the algal-bacterial systems offer dual benefits: (i) mitigation of the greenhouse effect due to $CO_2$ intake to microalgal biomass and (ii) reduction of aeration burden because of the generation and consumption of oxygen[50]. According to Tchobanoglous et al.[51], typical wastewater treatment plants utilize more than half of the total energy in mechanical aeration. This problem can be avoided in algal-bacterial systems, because microalgae can provide oxygen to heterotrophic aerobic bacteria for BOD removal from wastewater. Thus, compared to conventional BNR processes, algal-bacterial process can avoid (at least reduce the amount of) the external supply of oxygen.

Bacteria can also release nitrogen and phosphorus; those are needed by microalgae in their growth process[52]. The assimilation of nitrogen and phosphorus by microalgae into their biomass is advantageous for nutrient removal from wastewater. The residual biomass harvested after the treatment can be used for multiple purposes: methane production, biodiesel production, as green fertilizer, or as a biosorbent for heavy metals[53,54].

### 4.2. Applications of co-culture to nutrients removal

The use of co-culture in wastewater treatment process is an environmental friendly approach due to internal $CO_2/O_2$ exchange as described earlier. And, the performance of nutrients removal can also be increased in co-culture system as compared to that in single culture systems. This can be achieved by selecting appropriate microalgal and bacterial strains which could have positive effects on the other member. Microalgae and bacteria in the co-culture can be present as pure strains or as a consortium (such as mixed microalgae, wastewater bacteria or activated sludge bacteria).

The mixture of *C. vulgaris* and activated sludge bacteria as co-culture enhanced the performance of nutrients removal, also provided some other advantages like the removals of COD and pathogens and the effective harvesting of microalgae by sedimentation[55]. Aziz and Ng[56] achieved 60-75% removal efficiency for nitrate and phosphate in activated sludge-algae reactor using pig farm and palm oil industrial wastewaters. Simultaneous removal of organic acids, nitrate, ammonia and phosphate was obtained from synthetic wastewater by a mixed culture of photosynthetic bacterium *Rhodobacter sphaeroides* and a green alga *C. sorokiniana*[57]. Munoz et al.[50] achieved 49-71% removal of acetonitrile nitrogen by a symbiotic consortium of *C. sorokiniana* and a mixed bacterial culture. Table 1 shows the removal efficiency of nitrogen and phosphorus by different co-culture consortiums.

Liang et al.[11] investigated the effect of combined system of *C. vulgaris* and *Bacillus licheniformis* on nutrients removal in flasks at 6-d experiment. Using synthetic wastewater, nitrogen and phosphorus removal efficiencies were 78% (from initial 20 mg N/L) and 92% (from initial 4 mg P/L), respectively. By adjusting the pH from acidic to neutral, the removal performances of nutrients improved to 86% and 93% for nitrogen and phosphorus, respectively. It indicates that nutrients removal performance of co-culture can be enhanced by creating more favorable environmental conditions. They also found that chlorophyll content in the microalgal cells was also increased after pH adjustment[11].

There are several reports on the removal of nutrients by the interaction of microalgae strains and wastewater bacteria. For example, an algal strain *Coelastrum microporum* (that was isolated from effluent of the wastewater treatment facility) was cultured with indigenous heterotrophic bacteria of municipal wastewater. Potential effects of photoperiod on the performance of wastewater treatment were examined in this study[58]. The removal efficiencies were 36, 65%, and 88% for nitrogen (from initial 40 mg N/L), and 40, 60%, and 88% for phospho-

**Table 1. Removal of Nitrogen and Phosphorus from Wastewaters Using Algal-bacterial Symbiotic Co-culture**

| Co-culture (microalgae / bacteria) | Wastewater | N removal (%) | P removal (%) | References |
|---|---|---|---|---|
| *C. vulgaris / A. brasilense* | Synthetic | 91 | 75 | [43] |
| *C. vulgaris / B. licheniformis* | Synthetic | 86 | 93 | [11] |
| *C. vulgaris / Pseudomonas putida* | Synthetic | 80 | 60 | [62] |
| *C. microporum* / wastewater bacteria | Municipal | 88 | 89 | [58] |
| *C. vulgaris* / wastewater bacteria | Municipal | 24 | 70 | [33] |
| *C. vulgaris* / wastewater bacteria | Municipal | 97 | 98 | [7] |
| *C. pyrenoidosa* / wastewater bacteria | Landfill | 95 | 95 | [59] |
| *Chlorella* sp. / EM-1 | Aquaculture | 77-100 | 86-100 | [69] |
| *E. virdis* / activated sludge | Piggery | 34-39 | 31-53 | [52] |
| *S. obliquus* / activated sludge | Piggery | 36 | 65 | [52] |
| *C. sorokiniana* / activated sludge | Piggery | 21-25 | 23-54 | [52] |
| *C. sorokiniana* / activated sludge | Swine | 99 | 86 | [60] |
| *C. sorokiniana* / anaerobic sludge | Agro-industrial | 83-95 | 58-81 | [70] |
| *Scenedesmus* sp. / anaerobic sludge | Starch | 89 | 80 | [61] |

rus (from initial 5 mg P/L) under dark/light cycles of 12 h : 12 h, 36 h : 12 h, and 60 h : 12 h, respectively. It indicated that nutrient removal in an algal-bacterial photobioreactor was mostly facilitated under sufficient illumination, suggesting that the control of photoperiod is an important parameter in the algal wastewater treatment[58].

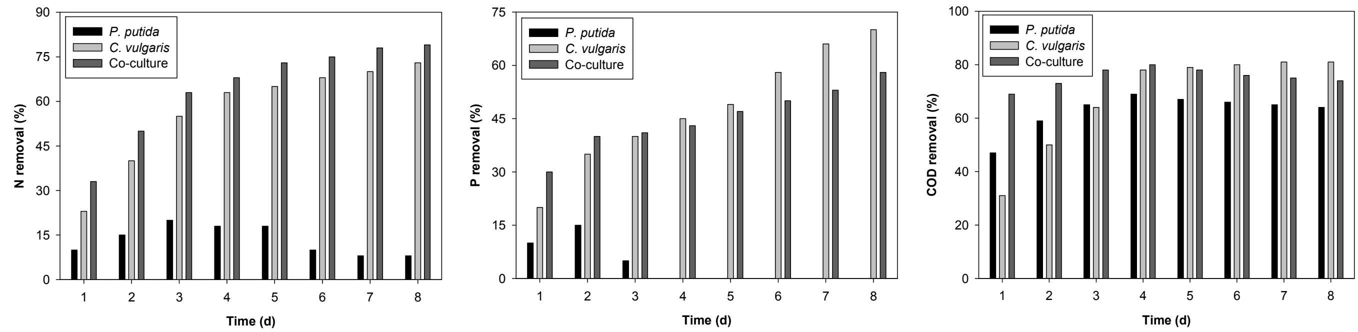
Ma et al.[33] treated municipal wastewater in flasks using the consortium of pure algal strain *C. vulgaris* and wastewater-borne bacteria and the optimized algal concentration. The results showed that initial algal concentration had an apparent effect on bacterial growth, and the presence of bacteria also had a substantial effect on algal growth process, indicating mutually symbiotic association between algae and bacteria at the initial stage of algae cultivation.

He et al.[7] checked the combined effect of *C. vulgaris* and wastewater bacteria on nutrients removal from municipal wastewater, and the algal-bacterial consortium resulted in the removal of 97% nitrogen and 98% phosphorus. This study also proved that microalgae play a dominant role in the removal of nitrogen and phosphorus, while bacteria remove most of the organic matters. The initial concentrations of nitrogen and phosphorus may also influence the uptake capability of microalgae. In another study using landfill leachate, the consortium of *Chlorella pyrenoidosa* and wastewater bacteria successfully removed 95% of nitrogen and phosphorus[59]. These studies showed that algae-bacteria consortium can be used significantly for the treatment of different kinds of wastewaters.

Microalgae can be co-cultured with activated sludge too, other than pure strains of bacteria and wastewater-borne bacteria. For example, de-Godos et al.[52] compared different microalgal species (such as *C. sorokiniana*, *Scenedesmus obliquus*, *Spirulina platensis*, and *Euglena viridis*) in symbiosis with activated sludge bacteria, and it was found that *S. obliquus* in the presence of activated sludge achieved highest removal of phosphorus (65%) while the consortium of *E. viridis* and activated sludge obtained the maximum nitrogen removal (39%) from piggery wastewater. There was no significant difference in the removal performances of these species, except *S. platensis* which were totally inhibited in piggery wastewater due to higher concentrations of nutrients[52]. Therefore, microalgal tolerance towards nutrients can be used as a key selection criterion. Using the swine slurry, *C. sorokiniana*-activated sludge consortium showed removal performances of 99% and 85% for nitrogen and phosphorus, respectively[60]. Using the co-culture of *Scenedesmus* sp. and anaerobic sludge, Ren et al.[61] achieved 89% of nitrogen removal and 80% of phosphorus removal from one type of starch wastewater.

Mujtaba et al.[62] performed nutrients removal from synthetic municipal wastewater using the co-culture of *C. vulgaris* and *Pseudomonas putida*, which is one of the aerobic bacteria popularly found in activated sludge systems. Figure 2 shows the removal of nitrogen by pure *P. putida* culture, pure *C. vulgaris* culture, and co-culture comprised of both. At 8-d treatment of synthetic wastewater, co-culture removed 80% nitrogen (from initial 50 mg N/L) which was higher than the performance by pure cultures. It indicates the positive symbiotic relationship exists between the microalga *C. vulgaris* and the bacterium *P. putida*. There is a possibility of nitrogen removal from the system through air stripping when pH is high. However, as the pH in the system was not so high to promote stripping, the uptake by biomass was the only mechanism for nitrogen removal.

The removal of phosphorus by pure cultures and co-culture of *C. vulgaris* and *P. putida* is also shown in Figure 2. During the treatment of first three days, co-culture was best in removing phosphorus from synthetic wastewater. After that, the removal efficiency was decreased as compared to that of pure *C. vulgaris* culture. The bacterium *P. putida* could release the stored phosphorus from its biomass into the culture medium under anaerobic environment. So, this may the reason for relatively less phosphorus removal in co-culture after three days. Overall, the phosphorus removal efficiency by co-culture was 60% (from initial 10 mg P/L) at the end of treatment (after 8 days). Phosphorus can be removed through precipitation under high pH. As




(a) (b) (c)

**Figure 2. Removal of (a) ammonium, (b) phosphate and (c) COD by pure cultures of microalga *C. vulgaris* and bacterium *P. putida*, and by their symbiotic co-culture.**

pH was low enough to avoid precipitation in the described system, biomass uptake was the main mechanism for phosphorus removal[62]. The results in Figure 2 showed that the co-culture system of *C. vulgaris* and *P. putida* exhibited better performance in both nutrients and COD removal than each of axenic cultures.

Using the co-culture system, the assimilation of nutrients into biomass provides further advantages because the residual biomass can be utilized later as green fertilizer due to the slow release of nutrients into the soil or as biosorbent for heavy metals[18]. According to Kawai et al.[63], *Chlorella* sp. and *Scenedesmus* sp. (mostly used in wastewater treatment) are rich in proteins, minerals, and vitamins A and B, and their amino acids are comparable to the levels found in fish meal and soy bean.

#### 4.3. Utilization of cell immobilization

The harvesting of biomass after treatment processes is a real practical concern in wastewater treatment. Although there are many methods thorough which biomass can be separated from the treated water (such as centrifugation, sedimentation, filtration, or flocculation etc.), many of them are either energy intensive, less efficient, or can cause secondary pollution. As an alternative, if microorganisms are attached or immobilized in a matrix material prior to treatment, their separation from the effluent can be more convenient. Immobilization of a microorganism not only prevents the wash out of biomass in bioreactors, but also offers a greater degree of operational flexibility and the convenience in recovery[64]. Therefore, immobilized co-culture systems have been used for the removal of nutrients. For example, de-Bashan et al.[43] co-immobilized *C. vulgaris* and *A. brasilense* together in alginate beads, and achieved 91% and 75% of removal efficiencies for nitrogen and phosphorus, respectively. It indicates that microalgae still have potential to uptake nitrogen and phosphorus when they are immobilized. It further elaborates the importance of symbiotic-beneficial interaction between microalgae and bacteria even in co-immobilized state.

For immobilization purpose, the polymers of carrageenan, chitosan and alginate are often used in algal systems, and the use of alginate beads is more frequent due to its high diffusivity, low production hazards, low polymer costs, and a simple and fast immobilization process[65,66].

The system of algal-bacterial biofilm has been known to have a potential for nutrients removal. For instance, an algal-bacterial biofilm achieved 70% of nitrogen removal as compared to only 36% of single bacterial biofilm[67]. Boelee et al.[68] also reported that microalgal biofilms can be used to treat municipal wastewater and significantly remove nitrogen and phosphorus. In this way, harvesting problem for microalgae can be resolved and the burden of conventional settling tank in wastewater treatment processes will be greatly reduced.

## 5. Conclusions

The negative and positive effects of microalgae-bacteria consortium have been discussed. The growth inhibiting or promoting interactions between microalgae and bacteria are based on involved species and employed environmental conditions. In the context of wastewater treatment, positive association (symbiosis) is more important because nutrients removal performance of microalgae can be enhanced in the presence of bacteria. Bacteria, due to the release of polymeric substances, can stimulate the growth of microalgae and increase the uptake capability of microalgae for nitrogen and phosphorus. Sustainable process of nutrients removal is possible in co-culture system due to exchange of $O_2$ and $CO_2$. Photosynthetic oxygen is used in bacterial respiration for organics degradation, while $CO_2$, which is a byproduct from bacteria, is used in turn by microalgae as a carbon source for photosynthesis. Therefore, the simultaneous removal of BOD and nutrients is realized through use of mutually symbiotic co-culture in a single reactor system.

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