

METHODS OF PURPLE PHOTOTROPHIC BACTERIA CULTIVATION AND APPLICATIONS THEREOF

FIELD OF INVENTION

The present invention provides a method of cultivating purple phototrophic bacteria. The present invention also provides a biomass obtained from the method.

BACKGROUND

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With the global population continuing to rise, the demand for food becomes increasingly urgent, driving an upsurge in edible plant cultivation. However, during agricultural production, soil-borne plant pathogens pose a hazard to various aspects of agriculture, affecting crop health, yield, and overall soil quality. These microorganisms, including bacteria, fungi, nematodes, and viruses, can lead to plant diseases, reduce nutrient availability, and compromise the overall productivity of agricultural systems. Various methods, such as the application of fungicides and pesticides, have been attempted to control soil pathogens. Nevertheless, this approach is increasingly scrutinized due to environmental concerns and the potential development of resistant strains. Therefore, it is crucial to develop effective and sustainable methods for controlling soil pathogens and improving agricultural outcomes.

In recent years, beneficial microorganisms, such as certain bacteria or fungi, can be introduced to the soil to outcompete or directly attack harmful pathogens, providing a natural form of control. Among them, *Rhodopseudomonas palustris*, a type of purple phototrophic bacteria (PPB), shows its superiority for improving agricultural production (Sakarika et al., 2020. Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. *Microb Biotechnol* 13(5), 1336-1365). PPB has the capability to utilize a wide range of substrates, both organic and inorganic compounds, owing to their versatile metabolic functions (Brown et al., 2022. *Rhodopseudomonas palustris*: A biotechnology chassis. *Biotechnol Adv*, 108001). Meanwhile, they can convert low-value substrates into high-value products, such as phytohormones (indole-3-acetic acid) and vitamins, which can promote plant growth. Furthermore, 5-aminolevulinic acid (5-ALA), which can act as antimicrobial substances, can be produced (Capson-Tojo et al., 2020. Purple phototrophic bacteria for resource recovery: Challenges and opportunities. *Biotechnol Adv* 43,

107567.; Dhar et al., 2023. Anoxygenic phototrophic purple non-sulfur bacteria: tool for bioremediation of hazardous environmental pollutants. World J Microbiol Biotechnol 39(10), 283). Conventional cultivation of *Rhodopseudomonas palustris* is expensive and involves a complex operational process due to the requirements of strict sterilization and synthetic/sterile substrates. Meanwhile, the slow growth of biomass limits its wide application. Despite the increasing interest in PPB for plant growth, currently, there is no existing approach to develop a low-cost PPB cultivation method and its application for supporting plant growth in practice.

Thus, there is a need for alternative and/or improved methods of cultivating purple phototrophic bacteria (PPB).

SUMMARY

Aspects and embodiments of the current invention will now be described by reference to the following numbered clauses.

1. A method of cultivating purple phototrophic bacteria (PPB), the method comprising the steps of:
 - (a) feeding a mixed bacterial population comprising PPB and a wastewater into a tubular reactor;
 - (b) circulating the bacterial population and wastewater in the tubular reactor under near-infra red (NIR) irradiation for a period of time and harvesting a portion of a resulting biomass; and
 - (c) repeating step (b).
2. The method according to clause 1, wherein the tubular reactor in its initial state before step (a) has not been sterilized.
3. The method according to clause 1 or clause 2, wherein the tubular reactor has a surface to volume ratio of from 20 to 70 m²/m³, such as from 30 to 50 m²/m³, such as from 33.3 to 40 m²/m³.
4. The method according to any one of the preceding clauses, wherein the light density of the NIR in the tubular reactor is from 10 to 40 W/m², such as from 15 to 30 W/m², such as about 21 W/m².

5. The method according to any one of the preceding clauses, wherein the purple phototrophic bacteria (PPB) comprises *Rhodopseudomonas palustris*, optionally wherein, after operation of the method for from 5 to 7 days, the *Rhodopseudomonas palustris* has a relative abundance of from 50 to 90%, such as from 70 to 85%, such as about 80% of the total bacterial population.

6. The method according to any one of the preceding clauses, wherein the wastewater is a food-processing wastewater, optionally wherein the food-processing wastewater comprises soybean wastewater.

7. The method according to any one of the preceding clauses, wherein one or more of the following apply:

- (i) the tubular reactor comprises from 5 to 30 tubular vessels, such as from 10 to 14 horizontal tubular vessels, optionally wherein the tubular vessels are connected together in series;
- (ii) each tubular vessel has a diameter of from 10 to 100 mm, such as from 20 to 60 mm, such as from 28 to 60 mm; and
- (iii) each tubular vessel has a length of from 500 to 5,000 mm, such as from 1,000 to 2,800 mm.

8. The method according to any one of the preceding clauses, wherein the wavelength of the near-infrared (NIR) irradiation is from 800 to 1,000 nm, such as about 850 nm.

9. The method according to any one of the preceding clauses, wherein the period of time is 5 to 7 days.

10. The method according to any one of the preceding clauses, wherein the method is operated in a semi-continuous mode with a hydraulic retention time (HRT) of from 10 to 40 days, such as from 15 to 30 days, such as about 20 days, optionally wherein a cleaning operation is performed from one to three times per HRT period to dislodge bacteria attached to an internal wall of the tubular reactor.

11. The method according to any one of the preceding clauses, wherein one or more of the following apply:

- (ai) a total chemical oxygen demand (TCOD) of from 5 to 20 g/L, such as from 6 to 15 g/L, such as about 7 g/L, such as 7.04 g/L;

- (aii) a soluble chemical oxygen demand (SCOD) of from 0.01 to 10 g/L, such as from 0.05 to 7 g/L, such as from 4.2 to 5 g/L, such as about 4.28 g/L, such as from 0.1 to 0.5 g/L, such as about 0.1 g/L;
- (aiii) step (b) has an organic loading rate of from 0.1 to 5 g COD/L/d, such as from 0.5 to 2 g COD/L/d, such as about 0.56 g COD/L/d;
- (aiv) a nitrogen concentration provided from ammonia ($\text{NH}_4^+\text{-N}$) of from 10 to 200 mg/L, such as from 20 to 100 mg/L, such as from 25 to 50 mg/L, such as about 28 mg/L;
- (av) a phosphorous concentration provided from phosphate ($\text{PO}_4^{3-}\text{-P}$) of from 50 to 200 mg/L, such as from 70 to 100 mg/L, such as about 72.97 mg/L;
- (avi) a biomass concentration before harvesting of at least 2.0 g total soluble solids (TSS)/L;
- (avii) a biomass yield of from 0.5 to 1 g total solids/g of consumed COD; and
- (aviii) a ratio of COD consumption to ammonia of from 10:1 to 100:1, such as from 20:1 to 40:1, such as about 30:1.

12. The method according to any one of the preceding clauses, wherein the biomass obtained from step (b) comprises one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid, optionally wherein the biomass obtained from step (b) comprises 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

13. A biomass obtained from a method according to any one of clauses 1 to 12, wherein the biomass comprises one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid, optionally wherein the biomass obtained from step (b) comprises 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

14. The biomass according to clause 13, wherein the biomass is provided in a dried form or as a bacterial solution.

15. A method of cultivating plants, the method comprising:

- (bi) providing a biomass obtainable according to any one of clauses 1 to 12 or a biomass according to clause 13 or clause 14 and plants in a soil or liquid medium; and
- (bii) adding the biomass to one or both of the plant and soil or liquid medium to enhance the growth rate of the plants.

16. Use of a biomass obtainable according to any one of clauses 1 to 12 or a biomass according to clause 13 or clause 14 for enhancing a growth rate of plants.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a photograph of a lab-scale tubular photoreactor setup (10L) according to Example 1 of the present disclosure.

FIG. 2 includes (a) a photograph of a pilot-scale tubular photoreactor setup (250L), (b) graph of Soluble Chemical Oxygen Demand (SCOD) (mg/L) of the influent and effluent against time (day) and (c) bacterial species abundance in the photoreactor according to Example 2 of the present disclosure.

FIG. 3 depicts the cultivation results of (a) three-colored amaranth and (b & c) rice by adding PPB solution according to Example 3 of the present disclosure. In Fig. 3(c) CK refers to Hoagland nutrient solution, T1 refers to Hoagland nutrient solution + 5% PPB (volume ratio) and T2 refers to Hoagland nutrient solution + 10% PPB (volume ratio).

DESCRIPTION

It has been surprisingly found that one can obtain a method to reduce the cost of purple phototrophic bacteria (PPB) cultivation and improve the biomass production by feeding a mixed bacterial population comprising PPB and a wastewater into a tubular reactor, circulating the bacterial population and wastewater in the tubular reactor under energy-saving near-infrared (NIR) light-emitting diodes (LEDs, e.g., 850 nm) and harvesting a portion of the resulting biomass. Most surprisingly, non-sterile conditions may be used in the process, significantly reducing the cost of PPB cultivation when compared to conventional processes which require sterilization of the system to be used. Furthermore, food-processing wastewater may be utilized as a substrate instead of synthetic substrates, further reducing the cost. A tubular reactor with a high surface-to-volume ratio may be used to enhance the biomass production. The harvested biomass may also find utility in plant cultivation, enhancing the growth rate of plants as demonstrated in the Examples disclosed herein.

Thus, in a first aspect of the invention, there is provided a method of cultivating purple phototrophic bacteria (PPB), the method comprising the steps of:

(a) feeding a mixed bacterial population comprising PPB and a wastewater into a tubular reactor;

- (b) circulating the bacterial population and wastewater in the tubular reactor under near-infra red (NIR) irradiation for a period of time and harvesting a portion of a resulting biomass; and
- (c) repeating step (b).

In embodiments herein, the word “comprising” may be interpreted as requiring the features mentioned, but not limiting the presence of other features. Alternatively, the word “comprising” may also relate to the situation where only the components/features listed are intended to be present (e.g. the word “comprising” may be replaced by the phrases “consists of” or “consists essentially of”). It is explicitly contemplated that both the broader and narrower interpretations can be applied to all aspects and embodiments of the present invention. In other words, the word “comprising” and synonyms thereof may be replaced by the phrase “consisting of” or the phrase “consists essentially of” or synonyms thereof and *vice versa*.

The phrase, “consists essentially of” and its pseudonyms may be interpreted herein to refer to a material where minor impurities may be present. For example, the material may be greater than or equal to 90% pure, such as greater than 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a condensation polymer” includes mixtures of two or more such compositions, reference to “the catalyst” includes mixtures of two or more such catalysts, and the like.

When used herein, the term “tubular reactor” refers to any suitable reactor for cultivating PPB that is tubular or substantially tubular in shape. In certain embodiments, the tubular reactor in its initial state before step (a) may not have been sterilized (for example, by autoclaving, by solvent (such as ethanol or isopropanol), chemical sterilization or by UV, X-ray or gamma irradiation). Advantageously, this reduces the cost of cultivating purple phototrophic bacteria when compared to conventional processes which require sterilization.

The tubular reactor may have any suitable surface to volume ratio. However, it is believed that having a high surface to volume ratio (e.g. $>10 \text{ m}^2/\text{m}^3$) may be beneficial, as this may enable increased interaction between the bacterial population and the NIR light. In certain embodiments, the tubular reactor may have a surface to volume ratio of from 20 to $70 \text{ m}^2/\text{m}^3$, such as from 30 to $50 \text{ m}^2/\text{m}^3$. In certain exemplary embodiments, the tubular reactor may have

a surface to volume ratio of from 33.3 to 40 m²/m³, such as about 33.3 m²/m³ or about 40 m²/m³. Advantageously, the high surface to volume ratio increases the photosynthetic efficiency of the purple phototropic bacteria, which in turn increases the biomass yield.

The term “about” as used herein can allow for a degree of variability in a value or range, for example, within 10%, within 5%, within 1%, within 0.5%, within 0.1%, within 0.05%, within 0.01%, within 0.005%, or within 0.001% of a stated value or of a stated limit of a range, and includes the exact stated value or range.

The tubular reactor may be configured as a single tubular vessel, but this may be impractical for larger-scale reactions and also for manufacturing such a vessel if one wants to have it occupy a smaller footprint than a linear tube would (e.g. by having the tube bend over itself). With that in mind, the tubular reactor may be formed from a plurality of tubular vessels. These vessels may be connected together in series or may be run separately in parallel to one another, or a combination of both. In certain embodiments, the plurality of tubular vessels may be connected together in series. In certain embodiments that may be mentioned herein, the tubular reactor may comprise from 5 to 30 tubular vessels, such as from 10 to 14 horizontal tubular vessels. As noted above, these tubular vessels may be connected together in series. As will be appreciated, the interconnects can be any suitable interconnect allowing the functioning of the tubular reactor and are not particularly limited, provided that they connect the tubular vessels together.

Each tubular vessel, when used herein, forms parts of the tubular reactor. These tubular vessels may have any suitable diameter, which may be chosen to allow substantial penetration of the vessel by the NIR light. In this context, “substantial” may refer to the NIR light penetrating into the interior of the tubular vessels to a depth that enables the selection and growth of the desired PPB over the operating timeframe of the process. As will be appreciated, the actual diameter chosen will depend on the intensity of the light source in question and may therefore be varied in accordance with the reactor designer’s plans for the particular reactor. However, in certain embodiments that may be mentioned herein, each tubular vessel may have a diameter of from 10 to 100 mm, such as from 20 to 60 mm, such as from 28 to 60 mm.

The length of each tubular reactor is not particularly limited, except from practical considerations. For example, each tubular vessel may have a length of from 500 to 5,000 mm, such as from 1,000 to 2,800 mm.

Advantageously, these dimensions increase the biomass yield as well as the scalability of the method for industrial-scale applications (e.g., plant cultivation).

It will be appreciated that the tubular vessels (and therefore substantially the tubular reactor) should be constructed from a material that is as transparent as possible (e.g. >95%, such as >99%, such as >99.9% transparent) to NIR.

When used herein, the term “near-infra red (NIR)” refers to the near-infrared region of the electromagnetic spectrum (e.g., from 780 nm to 2500 nm, such as 850 nm). In certain embodiments, the wavelength of the near-infrared (NIR) irradiation may be from 800 to 1,000 nm. In certain exemplary embodiments, the wavelength of the NIR irradiation may be about 850 nm.

In order to provide sufficient growth and selection for PPB, the NIR should be supplied in a sufficient light density, which can readily be determined by the skilled person. Any suitable light density of NIR may be used. For example, the light density of the NIR in the tubular reactor may be from 10 to 40 W/m², such as from 15 to 30 W/m². In certain exemplary embodiments, the light density of the NIR in the tubular reactor may be about 21 W/m².

When used herein, the term “purple phototrophic bacteria (PPB)” refers to a class of Gram-negative proteobacteria that are phototrophic, capable of producing their own food via photosynthesis. They are pigmented with bacteriochlorophyll a or b, together with various carotenoids, which give them colours ranging between purple, red, brown, and orange.

In certain embodiments, the purple phototrophic bacteria (PPB) may comprise *Rhodopseudomonas palustris*. As demonstrated in the Examples section of the present disclosure, the *Rhodopseudomonas palustris* may have a relative abundance of from 50 to 90%, such as from 70 to 85%, such as about 80% of the total bacterial population after operation of the method for from 5 to 7 days.

When used herein, the term “wastewater” refers to any suitable wastewater that has been used in various applications (e.g., in a home, in a business, or as part of an industrial process, for example, an agriculture process) but may still be used for PPB cultivation. In certain embodiments, the wastewater may be a food-processing wastewater. In certain exemplary embodiments, the food-processing wastewater may comprise soybean wastewater. In certain preferred embodiments, the wastewater may comprise organic material, nitrogen (from ammonia) and phosphorus (from phosphates) for PPB growth.

As will be appreciated, the period of time for the method to be conducted may be determined by the skilled person based on their knowledge of the field and whether the cultivation has been deemed to be completed or not. In certain embodiments, the period of time may be from 5 to 7 days.

As demonstrated in the Examples section of the present disclosure, the method may be operated in a semi-continuous mode with a hydraulic retention time (HRT) of from 10 to 40 days, such as from 15 to 30 days, such as about 20 days. As will be appreciated, the HRT can be varied outside these values by an operator based on their experience. When used herein, the term "hydraulic retention time (HRT)" refers to the time the wastewater spends in the tubular reactor. In certain embodiments, a cleaning operation may be performed from one to three times per HRT period to dislodge bacteria attached to an internal wall of the tubular reactor.

In certain embodiments, one or more of the following may apply:

- (ai) a total chemical oxygen demand (TCOD) of from 5 to 20 g/L, such as from 6 to 15 g/L, such as about 7 g/L, such as 7.04 g/L;
- (a ii) a soluble chemical oxygen demand (SCOD) of from 0.01 to 10 g/L, such as from 0.05 to 7 g/L, such as from 4.2 to 5 g/L, such as about 4.28 g/L, such as from 0.1 to 0.5 g/L, such as about 0.1 g/L;
- (a iii) step (b) may have an organic loading rate of from 0.1 to 5 g COD/L/d, such as from 0.5 to 2 g COD/L/d, such as about 0.56 g COD/L/d;
- (a iv) a nitrogen concentration provided from ammonia ($\text{NH}_4^+\text{-N}$) of from 10 to 200 mg/L, such as from 20 to 100 mg/L, such as from 25 to 50 mg/L, such as about 28 mg/L;
- (a v) a phosphorous concentration provided from phosphate ($\text{PO}_4^{3-}\text{-P}$) of from 50 to 200 mg/L, such as from 70 to 100 mg/L, such as about 72.97 mg/L;
- (a vi) a biomass concentration before harvesting of at least 2.0 g total soluble solids (TSS)/L;
- (a vii) a biomass yield of from 0.5 to 1 g total solids/g of consumed COD; and
- (a viii) a ratio of COD consumption to ammonia of from 10:1 to 100:1, such as from 20:1 to 40:1, such as about 30:1.

As will be appreciated, the parameters above are suggestions that can be varied outside of the ranges provided by a skilled operator should the need arise.

As will be appreciated, the various measurements of the wastewater (TCOD, SCOD, nitrate concentration, phosphorous concentration and biomass concentration etc.) may be readily

determined by the skilled person using conventional techniques and equipment, such as titration and UV-Vis spectrophotometry or commercial test kits/devices.

When used herein, the term “biomass” refers to the biological content in the reactors and may include materials produced by the growth of the mixed bacterial population, plus the bacteria themselves (e.g. PPB) and their metabolic products, such as hormones. In certain embodiments, the biomass obtained from step (b) may comprise one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid. In certain exemplary embodiments, the biomass obtained from step (b) may comprise 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

As demonstrated in the Examples section of the present disclosure, the purple phototrophic bacteria are found to be capable of secreting (plant) growth hormones. Thus, in a second aspect of the invention, there is provided a biomass obtained from the method disclosed hereinbefore, wherein the biomass comprises one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid. In certain exemplary embodiments, the biomass may comprise 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

In certain embodiments, the biomass may be provided in a dried form or as a bacterial solution. As will be appreciated, the exact preparation method to provide the biomass in the dried form or bacterial solution is not particularly important and will be readily apparent to a person skilled in the art.

As demonstrated in the Examples section of the present disclosure, the purple phototrophic bacteria are found to be capable of enhancing plant growth by supplying nutrients, secreting growth hormones, and enhancing stress resistance, as well as suppressing the growth of plant pathogens while increasing the abundance of probiotics. Thus, in a third aspect of the invention, there is provided a method of cultivating plants, the method comprising:

- (bi) providing a biomass obtainable according to the method disclosed hereinbefore and plants in a soil or liquid medium; and
- (bii) adding the biomass to one or both of the plant and soil or liquid medium to enhance the growth rate of the plants.

Further aspects and embodiments of the invention will now be discussed by reference to the following non-limiting examples.

EXAMPLES

Example 1 – PPB Cultivation (Lab scale performance (10L))

PPB can simultaneously assimilate organics, nitrogen, and phosphorus from waste streams via photoheterotrophic growth under anaerobic/illuminated conditions. With that, the efficiency of light absorption is a significant factor for biomass yield. A fermenter under continuous mode was successfully operated to cultivate PPB. However, the low surface-to-volume ratio of the fermenter (m^2/m^3) impeded the treatment capability and limited its biomass yield. This was due to low photosynthetic efficiency caused by the low surface area exposed to the light source. Thus, a tubular photoreactor with a high surface-to-volume ratio ($40 \text{ m}^2/\text{m}^3$) was designed for this application. The tubular photoreactor consisted of multiple horizontal tubular vessels, with a total working volume of 10 L as shown in FIG. 1. Raw wastewater collected from local food-processing plant was used to feed the photobioreactor. The total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), nitrogen concentration provided from ammonia ($\text{NH}_4^+\text{-N}$) and phosphorous concentration provided from phosphate ($\text{PO}_4^{3-}\text{-P}$) of the wastewater were 7.04 g/L, 4.28 g/L, 28 mg/L and 72.97 mg/L, respectively. A total of 0.8 L of the digestate was withdrawn and replaced with an equal volume of fresh 0.8 L raw wastewater daily, leading to the overall organic loading rate (OLR) of 0.56 g COD/L/d. The results showed that the reactor biomass concentration was stable at 2.0 g total suspended solids (TSS)/L with biomass yield of $\sim 0.7 \text{ g total solids (TS) / g of consumed COD}$. The OLR of the tubular reactor was much higher than that using other types of reactors, showing its superiority to cultivate PPB biomass.

Example 2 – PPB Cultivation (Pilot scale performance (250 L))

The setup of the PPB pilot system, as shown in FIG. 2a, included a 200-liter tubular photoreactor for cultivating PPB. This reactor comprised 14 stacked tubes (dimensions: 60 mm diameter x 2800 mm length), a 50-liter buffer tank, and a programmable logic controller (PLC) control panel. The surface to volume ratio of the tubular photoreactor was about $33.3 \text{ m}^2/\text{m}^3$. The reactor uses an infrared (IR) light source with a wavelength of 850 nm with a light density of 21 W/m^2 to support its operation and PPB enrichment. The reactor operated in a

semi-continuous mode with a hydraulic retention time (HRT) of 20 days. Due to the phototactic nature of PPB, which caused them to grow on the walls of the reactor, the attached biomass was periodically removed by wiping the inner surface once per HRT. During the initial days of operation, the soluble chemical oxygen demand (SCOD) of the effluent fluctuated but gradually stabilized. After 100 days, the SCOD remained consistent at approximately 100 mg/L, as depicted in FIG. 2b. The ratio of COD consumption to ammonium was about 30:1. As illustrated in FIG. 2c, *Rhodopseudomonas palustris* was the dominant species in the reactor, reaching a relative abundance of over 80%. Overall, the pilot-scale reactor demonstrated stable operation, effective PPB enrichment, and consistent biomass production.

Example 3 – Plant Cultivation

PPB are increasingly used in various fields, such as soil remediation, wastewater treatment, aquaculture, and energy recovery. However, there is limited research on their role in promoting plant growth. To fill this gap, it is hypothesized that PPB function as plant growth-promoting rhizobacteria (PGPR), capable of supplying nutrients, secreting growth hormones, and enhancing stress resistance.

Phytohormones contents were detected by MetWare (<http://www.metware.cn/>) based on the AB Sciex QTRAP 6500 LC-MS/MS platform. As shown in Table 1, the major plant hormones found in dried PPB biomass and bacterial solutions included 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde. The presence of these hormones indicates PPB's potential to significantly enhance plant growth.

Table 1. Major plant hormones in the dried biomass and bacteria solution.

Compounds	Class	Dried biomass (ng/g)	Bacteria solution ng/mL
2-oxindole-3-acetic acid	Auxin	13888.52	475.04
Indole-3-acetic acid	Auxin	4848.26	9.14
L-tryptophan	Auxin	3722.59	23.61
L-Phenylalanine	Salicylic acid	1703.15	86.22
Indole-3-carboxaldehyde	Auxin	1135.44	0.37

Several trials were conducted using PPB in plant cultivation, including for lettuce, *three-colored amaranth*, and rice. For soil culture, 20 mL of PPB solution was added to 1 L of soil or 1.1 L of PPB solution for a 1-hectare farm. For hydroponic culture, PPB solution was added to obtain 5% volume ratio.

For promoting lettuce growth: Two different operational methods were employed. In one method, PPB was scattered on the soil, while in the other method, microbes were sprayed onto the leaves and roots. Results showed that PPB addition increased overall edible plant growth performance, including the yield and quality of edible plant biomass. The analysis of microbial communities located in the plant roots revealed that plant pathogens, such as *Pantoea ananatis*, could be effectively suppressed, and the relative abundances of some probiotics, such as *Pseudomonas mosselii* and *Bacillus velezensis*, increased. In comparison to the control groups, the present methods demonstrated overwhelming superiority in terms of cost and edible plant growth performance. That is, this biomass can not only provide the common nutrients which can slowly be released and fertilize the soil, but also produce the added value of containing substances that promote plant growth, which conventional fertilizers do not have. This suggests that PPB biomass cultivated using the present methods has the potential to be developed into probiotics.

For promoting *Three-colored amaranth* and rice growth: Compared to the treatments with chemical fertilizer, the PPB-inoculated treatments exhibited considerable performance on the growth of *Three-colored amaranth*. This result highlights that the application of PPB increased the yield of *Three-colored amaranth* and shortened its growth period (see FIG. 3(a). Moreover, the PPB-inoculated treatments promoted the growth of rice with higher content of chlorophyll (FIG. 3(b) and 3(c)).

Conclusion

The present method can convert low-quality wastewater to high quality valuable-added products (in the form of probiotics) instead of sludge that requires additional cost for disposal. This not only treats the wastewater but also creates commercially viable products, making it an economically feasible solution. The method according to the present disclosure results in the production of new and dependable products that are in alignment with and supportive of the sustainable development goals of Singapore. It is important to note that the application of this technology as probiotics is still in its early stages, and further exploration for each specific application is an iterative process that requires a strong collaboration between research and industry.

CLAIMS

1. A method of cultivating purple phototrophic bacteria (PPB), the method comprising the steps of:
 - (a) feeding a mixed bacterial population comprising PPB and a wastewater into a tubular reactor;
 - (b) circulating the bacterial population and wastewater in the tubular reactor under near-infra red (NIR) irradiation for a period of time and harvesting a portion of a resulting biomass; and
 - (c) repeating step (b).
2. The method according to Claim 1, wherein the tubular reactor in its initial state before step (a) has not been sterilized.
3. The method according to Claim 1 or Claim 2, wherein the tubular reactor has a surface to volume ratio of from 20 to 70 m²/m³, such as from 30 to 50 m²/m³, such as from 33.3 to 40 m²/m³.
4. The method according to any one of the preceding claims, wherein the light density of the NIR in the tubular reactor is from 10 to 40 W/m², such as from 15 to 30 W/m², such as about 21 W/m².
5. The method according to any one of the preceding claims, wherein the purple phototrophic bacteria (PPB) comprises *Rhodopseudomonas palustris*, optionally wherein, after operation of the method for from 5 to 7 days, the *Rhodopseudomonas palustris* has a relative abundance of from 50 to 90%, such as from 70 to 85%, such as about 80% of the total bacterial population.
6. The method according to any one of the preceding claims, wherein the wastewater is a food-processing wastewater, optionally wherein the food-processing wastewater comprises soybean wastewater.
7. The method according to any one of the preceding claims, wherein one or more of the following apply:
 - (i) the tubular reactor comprises from 5 to 30 tubular vessels, such as from 10 to 14 horizontal tubular vessels, optionally wherein the tubular vessels are connected together in series;

(ii) each tubular vessel has a diameter of from 10 to 100 mm, such as from 20 to 60 mm, such as from 28 to 60 mm; and

(iii) each tubular vessel has a length of from 500 to 5,000 mm, such as from 1,000 to 2,800 mm.

8. The method according to any one of the preceding claims, wherein the wavelength of the near-infrared (NIR) irradiation is from 800 to 1,000 nm, such as about 850 nm.

9. The method according to any one of the preceding claims, wherein the period of time is 5 to 7 days.

10. The method according to any one of the preceding claims, wherein the method is operated in a semi-continuous mode with a hydraulic retention time (HRT) of from 10 to 40 days, such as from 15 to 30 days, such as about 20 days, optionally wherein a cleaning operation is performed from one to three times per HRT period to dislodge bacteria attached to an internal wall of the tubular reactor.

11. The method according to any one of the preceding claims, wherein one or more of the following apply:

(ai) a total chemical oxygen demand (TCOD) of from 5 to 20 g/L, such as from 6 to 15 g/L, such as about 7 g/L, such as 7.04 g/L;

(aii) a soluble chemical oxygen demand (SCOD) of from 0.01 to 10 g/L, such as from 0.05 to 7 g/L, such as from 4.2 to 5 g/L, such as about 4.28 g/L, such as from 0.1 to 0.5 g/L, such as about 0.1 g/L;

(aiii) step (b) has an organic loading rate of from 0.1 to 5 g COD/L/d, such as from 0.5 to 2 g COD/L/d, such as about 0.56 g COD/L/d;

(aiv) a nitrogen concentration provided from ammonia ($\text{NH}_4^+\text{-N}$) of from 10 to 200 mg/L, such as from 20 to 100 mg/L, such as from 25 to 50 mg/L, such as about 28 mg/L;

(av) a phosphorous concentration provided from phosphate ($\text{PO}_4^{3-}\text{-P}$) of from 50 to 200 mg/L, such as from 70 to 100 mg/L, such as about 72.97 mg/L;

(avi) a biomass concentration before harvesting of at least 2.0 g total suspended solids (TSS)/L;

(avii) a biomass yield of from 0.5 to 1 g total solids/g of consumed COD; and

(aviii) a ratio of COD consumption to ammonia of from 10:1 to 100:1, such as from 20:1 to 40:1, such as about 30:1.

12. The method according to any one of the preceding claims, wherein the biomass obtained from step (b) comprises one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid, optionally wherein the biomass obtained from step (b) comprises 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

13. A biomass obtained from a method according to any one of Claims 1 to 12, wherein the biomass comprises one or more of 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and, more particularly, 5-aminolevulinic acid, optionally wherein the biomass obtained from step (b) comprises 2-oxindole-3-acetic acid, indole-3-acetic acid (IAA), L-tryptophan, L-phenylalanine, and indole-3-carboxaldehyde and 5-aminolevulinic acid.

14. The biomass according to Claim 13, wherein the biomass is provided in a dried form or as a bacterial solution.

15. A method of cultivating plants, the method comprising:

- (bi) providing a biomass obtainable according to any one of Claims 1 to 12 or a biomass according to Claim 13 or Claim 14 and plants in a soil or liquid medium; and
- (bii) adding the biomass to one or both of the plant and soil or liquid medium to enhance the growth rate of the plants.

16. Use of a biomass obtainable according to any one of Claims 1 to 12 or a biomass according to Claim 13 or Claim 14 for enhancing a growth rate of plants.