Cultıvatıon of protococcales on wastewater

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Abstract. Many farms in Uzbekistan, Ukraine, Kazakhstan and other republics arrange the production cultivation of chlorella and other microalgae in special installations and the use of suspensions as a protein-vitamin additive in the ration of farm animals. The use of chlorella and other microalgae in the fattening of cattle, pigs and poultry allows for additional weight gain, the elimination of avitaminosis diseases and improvement of product quality.

1 Introduction

Chlorella and Stendesmus are valuable food and biological stimulants for mulberry silkworm caterpillars. Feeding them with silkworm leaves soaked in algae suspension increases cocoon weight by 15-20% and silkworm sheath weight by 30-40%.

In algae cultivation, pure chemical reagents and factory-available carbon dioxide are used. These components in pure nutrient medium increase the cost of production somewhat. Production of one ton of dry chlorella biomass requires 1300-1750 kg of mineral salts and 3750-8000 m³ of carbon dioxide.

One of the ways to reduce the price of microalgae biomass is to cultivate it on industrial and agricultural waste, which is discharged into artificial water bodies after preliminary treatment.

In municipal wastewater after treatment in special treatment plants, BOD5 often reaches 12-20 mg 02/g. The discharge of untreated wastewater is the main cause of its contamination with various organic impurities. It is very difficult to regulate the biological treatment of wastewater in industrial wastewater treatment plants due to the short-term residence of the wastewater in these water bodies and the presence of various organisms.

2 Methods

Special sewage treatment plants, i.e., biological ponds, are used to treat wastewater. However, they are difficult to regulate due to the seasonality of their operation.

The protection of water bodies from pollution is one of the central issues of scientific research. The cultivation of algae on different waste streams has been well studied [1-3]. Some researchers [4] have grown microalgae in biological oxidation contact stabilisation ponds filled with municipal and waste water. G. G. Winberg et al. [5] studied the efficacy of wastewater self-purification in single nonflowing ponds. Some papers give information

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on the algoflora of the ponds of wastewater treatment plants in some cities [6]. The bactericidal effect of algae developed in biological ponds has been shown [7-10].

However, there is not enough material on the biochemical and physiological characteristics of cultivated algae on waste water and other organic wastes and media.

We set ourselves the following objectives: selection of algae species and strains for cultivation on wastewater, study of bioecological and physiological and biochemical features of algae during cultivation on wastewater, study of purification effect of algae 3 cultivation on industrial and municipal wastewater with prevention of possible secondary pollution of water environment with products of microalgae degradation and their excretions, development of methods of algae cultivation on domestic and industrial wastewater.

For algae cultivation, Tashkent and Ahangaran wastewater was used. Tashkent, Akhangaran, Fergana and Margilan. Algologically pure strains of Chlorella and Stedesmus were used for culture. Changes in the physicochemical composition of water before and after algae cultivation were determined according to the generally accepted methodology of hydrochemical analyses.

According to our studies, the BOD5 value in the sewage of Tashkent city varies from 60-400 mg 02/l, permanganate oxidation 25-1200 mg 02/l. The active reaction in these effluents is neutral or slightly alkaline.

Municipal wastewater consists of mixed wastewater from industrial enterprises and municipal and domestic wastewater. The common sewage system of Tashkent city, along with municipal and domestic sewage, constantly receives sewage from flour mills, silk mills, meat processing plants, canning plants, factories, confectionary factories and others that release in huge amounts organic wastes. Some industrial wastewaters contain benzol, phenol, oil products, tar, cyanide, lead, sulphates, chlorides, various paints, etc. The colour of the effluent is mostly brown; the smell is oil and faecal. The presence of dissolved oxygen is not detected. The dense residue is 1.2- 1.7 g/l.

In municipal sewage of small towns (Ahangaran) the content of dissolved oxygen is 2.5-3.0 mg/l, BOD5- 46.6-120.0 mg 02/l, active reaction is neutral and slightly alkaline.

Different species of algae have been found in the ponds of wastewater treatment plants in some cities of Uzbekistan, the development of which indicates the potential for microalgae cultivation in these effluents.

The importance of municipal and industrial wastewater in the mass culture of algae is determined by the richness of nitrogen, phosphorus, potassium and other elements necessary for plant nutrition as well as the content of biologically active substances that stimulate the growth and development of algae in culture. According to many researchers [11], wastewater is rich in various amino acids, vitamins (especially group B), enzymes and other physiologically active substances.

In order to identify mass species of algae, further isolate algologically pure strains from them and use them in mass culture on organic wastes, the algoflora of the main wastewater facilities of some cities in Uzbekistan (Tashkent, Ahangaran and Fergana-Margilan industrial unit, Central Fergana) were studied.

More than 80 species and varieties of algae with predominance of protococcal representatives were found in phytoplankton of the studied water bodies.

During the cold season there is no water bloom and the number of species in phytoplankton decreases. Algae biomass during winter months is insignificant, maximum increase is observed in July-August, when phytoplankton biomass reaches 0.2-0.3 g/l. Water bloom is a typical phenomenon for water bodies of biological ponds and other wastewater treatment plants. In the reservoirs of the central part of the Soviet Union.

In the central part of the Soviet Union, blue-green algae (Microcystis aeruginosa, M. pulverea, Aphanizo-manon flos-aquae, etc.) and in Uzbekistan, green algae (Chlorella, Scenedesmus, Ankistrodesmus, Chlamydomanas, etc.) cause water bloom.

In biological ponds designed for wastewater treatment, water blooming should be considered a positive phenomenon. The rapid growth of phytoplankton in biological ponds and other ponds of wastewater treatment plants is a sign of active treatment of wastewater from various organic impurities. However, algae may be a source of secondary pollution in the pond. Their dead remains and deposited live bodies often form a thick layer of algal deposits, which in biological ponds during the summer months often reach a thickness of 0.2-0.5 m or more.

Consequently, without removing planktonic organisms from the waste water and regular cleaning of the pond bottom from various sediments, biological ponds cannot serve as an effective treatment facility. From the mass species developing in the individual ponds of the wastewater treatment plant, we have identified algologically pure strains of protococcus and other algae that grow well on all mineral media with the addition of organic matter and are characterised by mesosaproductive properties (table 1).

Table 1. algologically pure strains of protococcus and other algae.

Strain	**Saprobity degree**
Chlorella vulgarlis- YA 1-6	
Chlorella pyrenoidosa-YA -1-1	- - Meso
Chlorella sp(p-1)h. Str.	Oligo
Scenedesmus obliquus—YA-2-6	
Scenedesmus obliquus—YA-2-7	Oligo
Scenedesmus acuminatus—YA-2-8	
Euglena sp. YA-5-1	- - Meso

Urban wastewater is stable in terms of its physico-chemical properties. Their composition varies quantitatively, while the qualitative content of the main nutrients for algae remains constant. The qualitative change of the main nutrients in the wastewater is within the concentration limits at which most species and strains of protococcal algae can grow and develop more or less normally.

3 Results and discussion

The relative constancy of the qualitative composition of wastewater and its acceptable concentration for phototrophic and mixotrophic organisms allow it to be used as a nutrient medium for algae.

In the first experiment (8-15.U 2022) on mass cultivation of algae on urban wastewater, a slight increase of inoculant cells in cultivators and their yellowing and formation of flakes on the surface of the culture liquid were observed. Experiments were carried out without feeding the algae culture with pure carbon dioxide. Yellowing of the algae cells mainly indicates the lack of carbon dioxide in the medium for photosynthesis. During the experiment, the release of ammonia was observed to have an inhibitory effect on the growth and development of the algae in the culture.

The next experiment (20-28.U 2022) was carried out taking into account environmental factors inhibiting algae growth and development at +18 +29°C and illumination of 18-82k lx. The amount of suspension in each cultivator (glass aquarium)-15l. Carbon dioxide (5%) was purged in a mixture with air at an exposure time of 1min. The algae productivity in wastewater with $C0_2$ was found to be significantly higher than in standard nutrient medium 04. Consequently, the amount of nutrients in wastewater is quite sufficient for algae growth and development in culture.

It can be seen that the main factor inhibiting algae growth and development on waste water is insufficient C02 in the culture medium, which can be compensated by carbon dioxide blowing during cultivation of algae in cultivators. Intensive mixing of the algae suspension and carbon dioxide feeding are necessary conditions for algae cultivation on waste water. Massive mixing of wastewater makes it possible to use wastewater as a writing medium for algae.

It should be noted that the productivity of Stedesmus when cultured on wastewater was almost 1.5-2 times higher than on standard 0.4 mineral medium. This is due to the resistance of the alga itself, the nature and amount of nutrients, etc. The reproduction coefficients of Stedesmus on sewage and mineral medium are similar and have the same value of 1.5-2.2.

We also studied the productivities of protococcal microalgae when cultured in a continuous flow method in wastewater in special plants.

For algae cultivation, large-scale plants are constructed, replacing expensive industrial facilities. Calculations have shown that the cost of building plants with capacity of 400 thousand m3 of suspension per day is much cheaper (about 500 thousand rubles) than the cost of biostation with similar capacity. In a plant for mass cultivation of algae, it is possible to obtain a suspension with a density of 80-100 million/ml cells or more. Separating 1 m^3 of suspension yields 3-4 kg of paste (75% moisture content) or 0.7-1 kg of dry mass.

After separation of the algae biomass, the purified, accurate water should be directed to storage ponds with thickets of higher aquatic plants (Lemna minor, L. gibba, Hydrilla verticullate, Potamogeton pusillus, Lizanis latifolia, Spirpus lacustris, Phragmites communis, Nastrutium fontanum, etc.). Some metabolic products of algae cells remain in the treated effluent, which is absorbed by macrophytes and thereby purifies the aquatic environment. In non-freezing water bodies, many aquatic macrophytes (reed, reedgrass, couch grass, bulrush, etc.) are vegetated all year round. However, to prevent possible secondary pollution of the aquatic environment through the decomposition of dead residues, regular cleaning of these thickets should be carried out.

Wastewater after secondary treatment in holding ponds can be used for irrigation and other purposes. Cultivation of algae on wastewater. This draining of wastewater, separation of biomass and its use for fodder and other purposes is the most cost-effective method of wastewater treatment.

There are also other ways of selling algae biomass grown on wastewater. This is the release of algae-enriched wastewater into fish ponds. Experiments have shown that regular application of chlorella and Stedesmus suspension to pond water increases the number of forage zooplankton species, suppresses sorrel species of algae, improves the hydrochemical regime of water bodies and increases their fish productivity by 20-40% [12]. Wastewater enriched with algal biomass is used for irrigation, as chlorella and Stedesmus are biostimulants for agricultural plants, and their biomass is a valuable fertilizer for cultivated fields. Regular application of chlorella and Stedesmus suspensions to cotton, rice, onion, maize and other crops increases yields by 12-25% [12].

The biochemical properties of algae grown on wastewater and those cultivated on pure mineral media are similar. In the dry biomass of chlorella and stendesmus, 39-50 % protein, 30-36 carbohydrates, 5-10 fat and 8-12 % mineral substances, 1200-2000 mg/kg carotene, 800-900mg/kg ascarbic acid were found, as well as the vitamins B-B1, B2, B6, B15, PP, K etc. were found. The content of carbohydrates (65%), especially starch (42%), increases when the algae is cultivated on waste water. In chlorella grown on municipal sewage the starch content is 2-2.5 times higher than on mineral "medium 04".

4 Conclusions

To obtain protein and other useful substances from algal biomass, the technological scheme recommended by Bulgarian researchers [13-15] can be used. The tray plants developed in the Department of Microbiology and in the Special Design Bureau "Silk" of the Department of Silkworms of the Ministry of Agriculture of Uzbekistan, with circulating algae suspension, should be used as cultivators. A single installation should accommodate the weight of the daily volume of wastewater from a town, village, or livestock complex. Thus, for example, to receive wastewater arriving at the Salar sewage treatment plant in Buzhora (300-400 thousand m^3/day), it is desirable to have 6-8 plants with a capacity of 350-400 thousand m^3 each. After receiving wastewater, algae is planted in them and their cultivation lasts for 5-6 days, i.e., until the culture reaches a plateau. Then it is passed through a separator, and the treated effluent is transferred to holding ponds. The plant, freed from suspension, is again filled with effluent and seeded with algae. In this way, the tanks are filled and emptied alternately for 5-6 days. Their capacity is divided by the daily volume of wastewater to be treated. It is not uncommon to leave part of the suspension in the stop and use it as a mother crop. However, it is recommended to have an active fresh slurry for seeding, which should be stored in a special container.

Thus, municipal wastewater can serve as a nutrient medium for the mass cultivation of protococcus and other green algae in special installations. Untreated and treated municipal wastewater can be used for cultivation. Many honest industrial effluents (from oil refineries and nitrogen and fertilizer plants), which are unfavourable for algae cultivation, can only be used for this purpose mixed with municipal wastewater.

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