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Potential of *Rhodobacter capsulatus* Grown in Anaerobic-Light or Aerobic-Dark Conditions as Bioremediation Agent for Biological Wastewater Treatments

Stefania Costa [1], Saverio Ganzerli [2], Irene Rugiero [1], Simone Pellizzari [2], Paola Pedrini [1] and Elena Tamburini [1,*]

[1] Department of Life Science and Biotechnology, University of Ferrara, Via L. Borsari, 46 | 44121 Ferrara, Italy; stefania.costa@unife.it (S.C.); irene.rugiero@unife.it (I.R.); pdp@unife.it (P.P.)

[2] NCR-Biochemical SpA, Via dei Carpentieri, 8 | 40050 Castello d'Argile (BO), Italy; S.Ganzerli@ncr-biochemical.it (S.G.); S.Pellizzari@ncr-biochemical.it (S.P.)

* Correspondence: tme@unife.it; Tel.: +39-053-245-5329

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Abstract: The use of microorganisms to clean up wastewater provides a cheaper alternative to the conventional treatment plant. The efficiency of this method can be improved by the choice of microorganism with the potential of removing contaminants. One such group is photosynthetic bacteria. *Rhodobacter capsulatus* is a purple non-sulfur bacterium (PNSB) found to be capable of different metabolic activities depending on the environmental conditions. Cell growth in different media and conditions was tested, obtaining a concentration of about 10^8 CFU/mL under aerobic-dark and 10^9 CFU/mL under anaerobic-light conditions. The biomass was then used as a bioremediation agent for denitrification and nitrification of municipal wastewater to evaluate the potential to be employed as an additive in biological wastewater treatment. Inoculating a sample of mixed liquor withdrawn from the municipal wastewater treatment plant with *R. capsulatus* grown in aerobic-dark and anaerobic-light conditions caused a significant decrease of N-NO_3 (>95%), N-NH_3 (70%) and SCOD (soluble chemical oxygen demand) (>69%), independent of the growth conditions. A preliminary evaluation of costs indicated that *R. capsulatus* grown in aerobic-dark conditions could be more convenient for industrial application.

Keywords: bioremediation; *Rhodobacter capsulatus*; wastewater treatment; NUR; OUR

1. Introduction

Wastewater treatment and reuse is not new, and knowledge on this topic has evolved and advanced in the past years. As the human population continues to grow and urbanize, the challenges for securing water resources and disposing of wastewater will increasingly demand new and sustainable technologies [1]. The general purpose of wastewater treatments is to remove pollutants that can harm the aquatic environment and natural ecosystems in case they are discharged. Because of the deleterious effects of the low concentration of dissolved oxygen in aquatic life, wastewater treatment has been historically focused on the removal of pollutants that would deplete dissolved oxygen and favor eutrophication in receiving waters [2]. Traditionally, both chemical and biological treatments or a combination of them have been employed [3]. However, biological treatments have many advantages because they are cheaper, more reliable, can be done on site and their efficiency can be improved by the choice of a variety of microorganisms [4]. Bioremediation processes explore the use of biological mechanisms to destroy, transform or immobilize environmental contaminants

to protect the environment. Living organisms are emerging as one of the most useful alternative technologies for restoring contaminated sites and removing contaminants from soils and waters [5]. Both aerobic and anaerobic microorganisms are used for treating wastewaters. One such group of potential microorganisms is the purple non-sulfur bacteria (PNSB), belonging to the broader family of purple phototrophic bacteria (PPB) [6]. They are Gram-negative bacteria, widely distributed in various habitats such as soil, freshwater and ocean, and can be readily isolated from these sources [7]. They are characterized by versatile metabolic activities including photoautotrophic activity, with light as the energy source, H_2 as the electron donor and CO_2 as the electron acceptor/carbon source; chemoheterotrophic growth in darkness, using organics as the electron donor/carbon source and O_2 as the electron acceptor; and photoheterotrophic activity, using some sugars and organic acids as the electron donors/carbon source, with light as the energy source [8]. Since the 1960s, the application of PSNB in wastewater bioremediation has been extensively studied, namely in treating olive mill wastewater, dairy wastewater, poultry slaughterhouse wastewater, seafood wastewater, palm oil mill effluent and domestic wastewater [9]. This method has provided high efficiency in removing COD (Chemical Oxygen Demand) and nitrogen compounds, as well as hydrogen sulphide [10]. The most studied species is *Rhodobacter spheroides*, but applications of *Rhodopseudomonas palustris*, *Rubrivirax gelatinosus*, *Rhodocyclus gelatinosus* and *Rhodovulum sulfidophilum* have been also reported [11–13]. Notwithstanding the promising evidence, PNSB-based technology has not been used in the large-scale industrial application of wastewater treatments, and even the most recent studies regard almost exclusively their potential as bio-hydrogen producers [14], or as generators of value-added products from wastewater fermentation. In fact, it is known that PNSB can convert organic carbon into biomass that can be used as a source of single-cell proteins, carotenoids, macromolecules and pantothenic acid for fertilizer or feed [15].

Rhodobacter capsulatus is a selected PNSB bacterium that has been used in the past for the investigation of the bacterial photosynthetic metabolism and related characteristics [16]. It stands out as a particularly interesting individual species as it can rapidly grow in several photosynthetic and dark modes [17]. Due to its capability of using nitrate as an electron acceptor and ammonium as a substrate [18], the role of *R. capsulatus* could be tested in both wastewater denitrification and nitrification processes, and not only for its denitrifying potential as is usually cited in the literature [19–21]. It is also hardy and stable for long-term storage under appropriate conditions [19], making it very attractive for industrial use. However, no application of *R. capsulatus* in wastewater treatment on a large scale has been so far reported.

Taking into account its proven versatile metabolic behavior depending on environmental conditions [22], the aim of this study was to compare *R. capsulatus* cell growth in anaerobic-light and aerobic-dark conditions, using appropriate culture media. Then, the two biomasses obtained were added as bioremediation agents in samples of mixed liquor withdrawn from a municipal wastewater treatment plant, both in aerobic (nitrification) and anoxic (denitrification) conditions. The capacity of nitrogen and COD removal was tested at a 1 L batch scale, and our results are reported below.

2. Materials and Methods

2.1. Bacterial Strain and Media

The strain of PSNB *Rhodobacter capsulatus* DSM 1710 (ATCC 11166) was purchased as lyophilized tab from the culture collection of Leibniz Institute DSMZ—German Collection of Microrganisms and Cell Cultures (Braunschweig, Germany). It is formerly known as *Rhodopseudomonas capsulata* [23]. For anaerobic-light (AN-LT) growth, a modified Rhodospirillaceae medium (MR medium) was used: yeast extract, 0.30 g/L (Difco); Na_2-succinate 1.00 g/L; (NH_4)-acetate 0.50 g/L; Fe(III)-citrate solution (0.1% in H_2O), 5.00 mL; KH_2PO_4, 0.50 g/L; $MgSO_4 \cdot 7H_2O$, 0.40 g/L; NaCl, 0.40 g/L; NH_4Cl, 0.40 g/L; $CaCl_2 \cdot 2H_2O$, 0.05 g/L; vitamin B12 solution (10 mg in 100 mL H_2O), 0.40 mL; trace element solution SL-6, 1.00 mL/L (see below); L-cysteinium chloride, 0.30 g/L; resazurin, (0.1%) 0.50 mL/L.

Trace elements solution SL-6: $ZnSO_4 \cdot 7H_2O$, 0.10 g/L; $MnCl_2 \cdot 4H_2O$, 0.03 g/L; H_3BO_3, 0.30 g/L; $CoCl_2 \cdot 6H_2O$, 0.20 g/L; $CuCl_2 \cdot 2H_2O$, 0.01 g/L; $NiCl_2 \cdot 6H_2O$, 0.02 g/L; $Na_2MoO_4 \cdot 2H_2O$, 0.03 g/L. Complex culture medium for aerobic-dark (AE-DK) growth was prepared mixing bacteriological peptone, 5 g/L and meat extract, 3 g/L, as suggested by Weaver [16]. The master cell bank was maintained at −20 °C in cryovials on 1 mL of MR medium, mixed with 0.5 mL of glycerol as crioprotectant agent. The working cell bank was conserved at 4 °C in MR medium-agar tubes for six months and used as seed cultures. All complex media were purchased from Difco (BD, Franklin Lakes, NJ, USA), whereas all chemicals were purchased from Fluka Analytical (Steinheim, Germany), unless otherwise stated.

2.2. Anaerobic-Light (AN-LT) Growth

First, 1 mL from a cryovial of *Rhodobacter capsulatus* cells was inoculated in sterile MR medium (9 mL; the medium was boiled for 15 min and bubbled with sterilized nitrogen gas). After 48 h the cell suspension was used to inoculate completely filled screw-cap Erlenmeyer flask containing 90 mL of sterile MR medium, then incubated in a light cabinet and exposed to a LED light, at 30 °C for 72 h. A slow agitation rate was maintained to assure homogenous illumination, and pH adjusted to 6.8. Samples were withdrawn every 12 h up to 72 h of fermentations, and analyzed for biomass content.

2.3. Aerobic-Dark (AE-DK) Growth

First, 1 mL from a cryovial *Rhodobacter capsulatus cells* was inoculated in the sterile medium containing bacteriological peptone and meat extract (9 mL). After 48 h the cell suspension was used to inoculate a sterile complex medium (90 mL) in 250 mL Erlenmeyer flask closing with cotton plug, and then incubated in dark at 30 °C for 72 h. The pH was adjusted to 7.0. The aerobic conditions were given by agitation at 120 rpm. Samples were withdrawn every 12 h up to 72 h of fermentations, and then analysed for biomass content.

2.4. Mixed Liquor Samples

Samples of mixed liquor was provided by the wastewater treatment plant of Bologna, a city in the North of Italy with about 500,000 inhabitants. The plant has an overall potential capacity of 900,000 inhabitants equivalents and consists of a series of unit processes that receive polluted raw sewage directly from the sewer system and progressively clean it to a point that it can be safely discharged to a receiving water. It includes a biological treatment with activated-sludge process and a downstream sewage treatment plant. Figure 1 shows a simplified flow diagram for biological processes used: the influent wastewater goes through stages of denitrification and nitrification in which different compound (namely, nitrogen and biodegradable COD) are removed out of the wastewater.

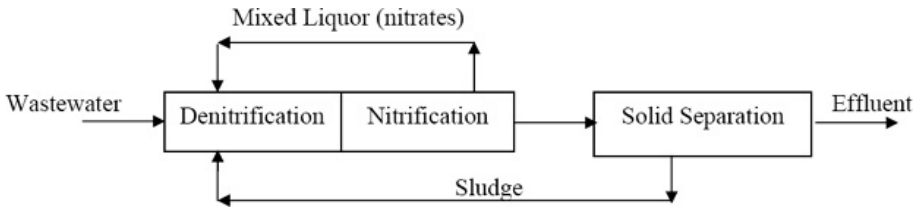


Figure 1. Simplified flow diagram of biological processes of the wastewater plant treatment of the city of Bologna, where samples used in this study were withdrawn.

Mixed liquor samples characteristics at the moment of withdrawal were as follows: MLSS (Mixed Liquor Suspended Solids), 3000 mg/L; soluble COD (Chemical Oxygen Demand), 74.10 mg/L; $N\text{-}NO_3$, 1.25 mg/L; $N\text{-}NO_2$, 0.08 mg/L; $N\text{-}NH_3$, 1.42 mg/L. All samples were stored and maintained at 4 °C for 24 h until analyzed and used for experiments. Before the tests, samples were sanitized in autoclave (120 °C for 15 min).

2.5. Nitrate Uptake Rate (NUR) and Oxygen Uptake Rate (OUR) Tests

In the NUR determination, two samples of mixed liquor from denitrification tank (250 mL) of municipal wastewater were diluted to 50% with distilled water and mixed with 0.75 g/L of CH_3COONa and 0.3 g/L KNO_3 in denitrification conditions (anoxia). One sample was inoculated with 100 ppm of *R. capsulatus* AN-LT grown, the other with 100 ppm of AE-DK grown. The decrease in N-NO_3 content was followed for 4 h and plotted against sampling time. The NUR (mg/gVSS·h) of the samples was obtained from the slope of the graph divided by the volatile suspended solid (VSS, mg/L). In the OUR measurement, two aliquots of 250 mL of mixed liquor from oxidation tank (nitrification) of municipal wastewater was taken, added with 0.75 g of CH_3COONa and aerated until saturation of Dissolved Oxygen (DO) (nitrification); then, the DO fall was continuously monitored by using an automatic probe (Mettler Toledo, Garvens, Germany). The OUR (mg/gVSS·h) of each sample equaled the slope of the DO depletion versus time divided by the volatile suspended solid (VSS, mg/L). All tests were carried out in triplicate against blank.

2.6. Batch Tests

All bioreactors were 1 L glass tanks. They were sanitized under steam stripping conditions (100 °C for 30 min), filled with 700 gr of sterile (120 °C for 15 min in autoclave) mixed liquor and inoculated with 100 mg/L of biomass suspension diluted to 10^7 CFU/mL. For inoculum, biomass was withdrawn in exponential phase from the cultivation tank, properly diluted and stored at 4 °C until inoculating bioreactors. A magnetic stirrer was used to mix biomass and mixed liquor homogeneously. Denitrification bioreactors were unaerated and oxygen levels kept at 0.4 mg/L. Nitrification bioreactors were aerated with an air flow of 90 L/h as to guarantee oxygen saturation.

Simulating denitrification of municipal wastewater, two different batches were set up as follows:

- Batch DM1: mixed liquor inoculated with 100 mg/L (10^7 CFU/mL) aerobic-dark (AE-DK) grown *R. capsulatus*
- Batch DM2: mixed liquor inoculated with 100 mg/L (10^7 CFU/mL) of anaerobic-light (AN-LT) grown *R. capsulatus*

Simulating nitrification of municipal wastewater, other two different batches were set up as follows:

- Batch NM1: mixed liquor inoculated with 100 mg/L (10^7 CFU/mL) of aerobic-dark (AE-DK) grown *R. capsulatus*.
- Batch NM2: mixed liquor inoculated with 100 mg/L (10^7 CFU/mL) of anaerobic-light (AN-LT) grown *R. capsulatus*.

In all cases, temperature was set at 30 °C and pH not controlled, mimicking as closest as possible what happens in industrial plant treatment. SCOD, N-nitric, N-nitrous, N-ammonia and biomass concentration were monitored and measured every 12 h.

2.7. Analytical Assays

Biomass concentration was determined as optical density at 600 nm (Shimadzu, Kyoto, Japan) and as *colony forming units* (UFC)/mL in Petri dishes, after 72 h of incubation on agar-MR medium or agar-complex medium at 30 °C, respectively. Supernatant, collected by centrifuging samples at 4500 rpm for 15 min, was used for SCOD, N-NO_3, N-NO_2 and N-NH_3 measurements, and following the ASTM standard methods [24]. All the experiments were carried out in triplicate to ensure the accuracy of data and the reported results were the average values.

3. Results

3.1. Comparison between R. capsulatus Cell Growth in Aerobic-Dark and Anaerobic-Light Conditions

Growth curves were obtained by plotting the cell concentration (CFU/mL) versus the time for both aerobic-dark and anaerobic-light conditions. Figure 2 shows the comparative profiles of the two growths, and it shows that when *R. capsulatus* was grown anaerobically in light, the maximum cell concentration reached an order of magnitude higher than that obtained in aerobic and dark conditions (3×10^9 versus 3×10^8 CFU/mL, respectively).

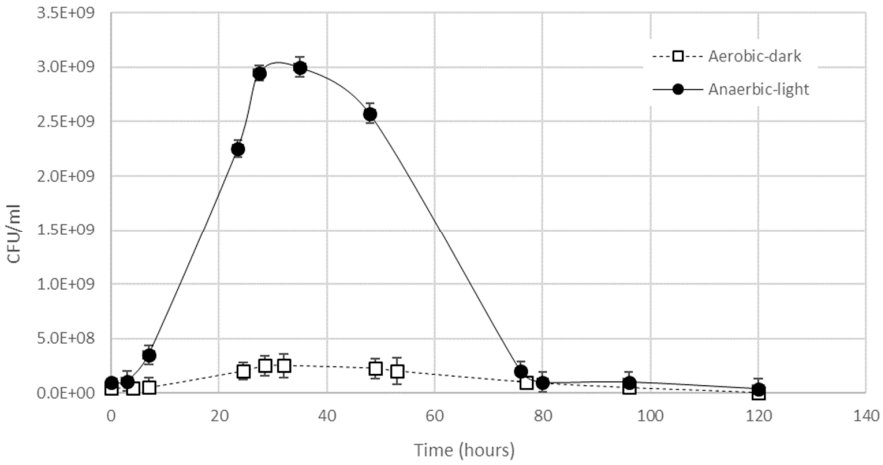


Figure 2. Experimental profile of growth curves for *R. capsulatus* grown in aerobic-dark and anaerobic-light conditions.

In both case, lag phases were conveniently short, and replication was terminated after 30 h, probably due to some substrate depletion. In the absence of external nutrient supplies, in the remaining 80 h, the anaerobically grown biomass lost an order of magnitude of CFU/mL. In these conditions, recovering the cell suspension by 28–30 h from the beginning of the process could assure the maximum viable cell concentration. Previous studies revealed that the standardized count that can be used to inoculate the media of biodegrading systems is within the range of 10^5–10^6 CFU/mL [25]. For anaerobic-light growth, the stationary phase was very short and in few hours kinetic death prevailed. In the exponential phase, *R. capsulatus* showed a doubling time of 19 h. For aerobic-dark growth the doubling time was 36 h, and up to 60 h, the cell concentration seemed not to decrease significantly. The optimal light intensity for biomass growth varies from microorganism to microorganism, and light also influenced the presence and the composition of the bacteriochlorophylls and carotenoids produced by the cells, which in turn has an effect on the cell suspension's color [26]. In this case, the biomass grown in chemotrophic conditions gave a dark pink to red color, probably due to the biosynthesis of lycopene, rhodopin, and spirilloxanthin, whereas in phototrophic conditions the color turned to brownish-red or greenish-brown, probably due to the presence of the spheroidenone series [27]. It is worth noting that, independent of the environment of growth, after several weeks of storage at room temperature in closed bottles, the cells' viability was 10^6–10^5 CFU/mL (data not shown).

3.2. NUR and OUR

Most activated sludge processes used for biological nitrogen removal have a mixed, non-aerated anoxic zone before the oxygenation tank, where no oxygen is present and nitrate reduction occurs [28]. Usually, the rate of substrate utilization has been observed to be lower when nitrate instead of oxygen is used as the electron acceptor, principally because not all of the endogenous heterotrophic bacteria present in the activated sludge system are facultative organisms capable of nitrate reduction [29].

Inoculating *R. capsulatus* could enhance the overall nitrogen uptake rate, while reducing the time of treatment and improving the efficiency of the process. We used the NUR test to evaluate the potential of the nitrogen removal rate with the external inoculum of *R. capsulatus* grown in different conditions. Mixed liquor from a municipal wastewater biological treatment plant was used as the soluble substrate for this experiment, as well as for OUR tests. In the NUR tests, nitrate and an external carbon source were added in excess to avoid nutrient depletion disguising the results. We found a significantly different denitrification rate depending on the type of biomass used as the inoculum (Figure 3).

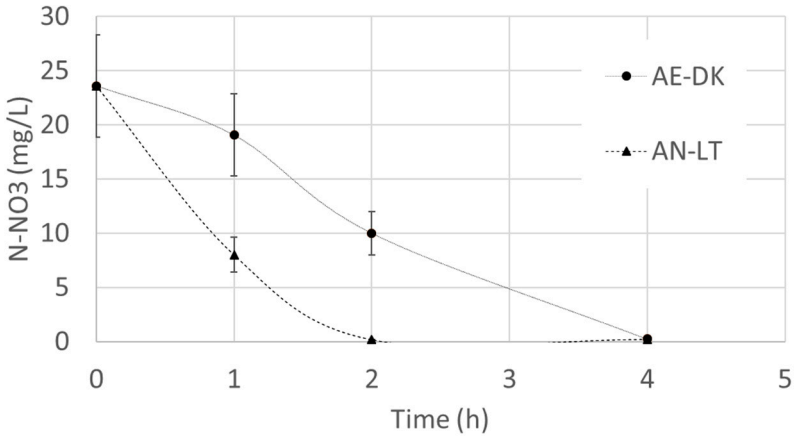


Figure 3. Nitrate depletion with time during NUR test for *R. capsulatus* grown in aerobic-dark (AE-DK) and anaerobic-light (AN-LT) conditions.

The inoculum of anaerobic-light (AN-LT) biomass almost permitted us to obtain a slope of depletion. When aerobic-dark (AE-DK) biomass was added, we observed a short lag phase at the beginning which could be due to the need for a period of adaptation to the anoxic environment by the cells. When excluding the first hour of the experiment from the calculation, the NO_3 consumption rate was similar to the one obtained with the AN-LT biomass. NUR values in the two cases are reported in Table 1. It is clear that in the presence of AN-LT biomass, NUR is more efficient, probably because in this case the biomass re-started growing in a similar environment of cell growth.

Table 1. NUR and OUR tests results.

Test	NUR (mg/gVSS·h)	OUR (mg/gVSS·h)
AE-DK	10.08 ± 2.75	6.20 ± 1.23
AN-LT	13.90 ± 2.67	4.73 ± 0.98

In the biotechnological field, microbial respiration is typically expressed as the oxygen uptake rate (OUR). Although OUR is a general term which does not presuppose any specific conditions in its determination, in the wastewater field it is usually reserved for on-line measurements of oxygen consumption through the measurement of the residual dissolved oxygen (DO). Indeed, OUR is often referred to as the dynamic respirometric index, as a measure of the aerobic degradation of organic material by aerobic microorganisms [30]. Furthermore, OUR can provide much information concerning the treatment plant's performance, wastewater characteristics, the degradability of special concentrated streams as well as the parameters needed to predict possible optimizations of a treatment plant. The OUR measurements proved that the oxygen-depletion bioprocesses in mixed liquor are a necessary but not sufficient condition for nitrification [31]. Figure 4 shows the respirograms of mixed liquor with the addition of *R. capsulatus* as the inoculum, both in AE-DK and AN-LT growth conditions. The OUR measurements can be performed in various ways which have been described in detail [32]. In this case, tests were carried out by adding a readily biodegradable substrate such as acetate to

avoid false-negative results. They could be caused by the excess of nitrogen due to the presence of human waste in the sludge, which could lead to an imbalance in the optimal COD:N ratio of 100:5 [33], or to the presence of inhibitory streams, or to an occurrence of endogenous, slowly biodegradable organic carbon. In fact, as it is well known while the carbon sources and oxygen have been consumed, DO progressively decreases due to the cells' respiration. It is worthwhile to note that the oxygen was completely depleted in 45 min. In this case, as expected, the AE-DK-grown biomass seemed to be better adapted to the oxygen-rich environment, contrary to the AN-LT biomass which showed a lag phase before starting aerobic respiration (Table 1).

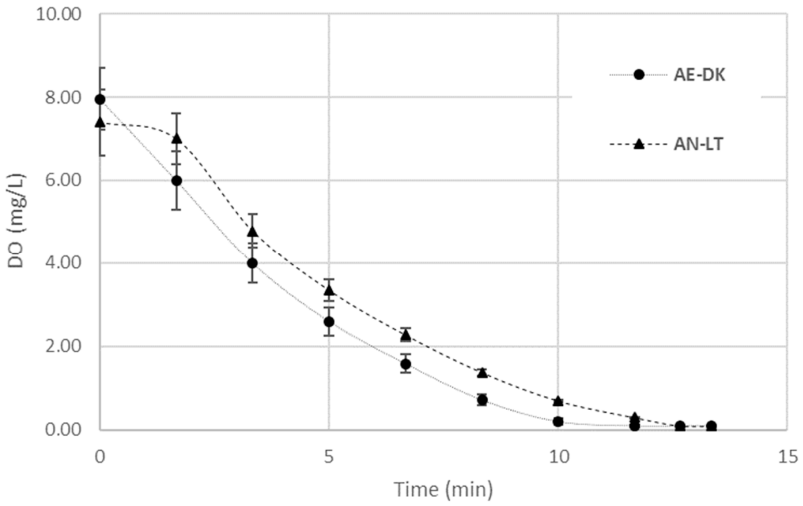


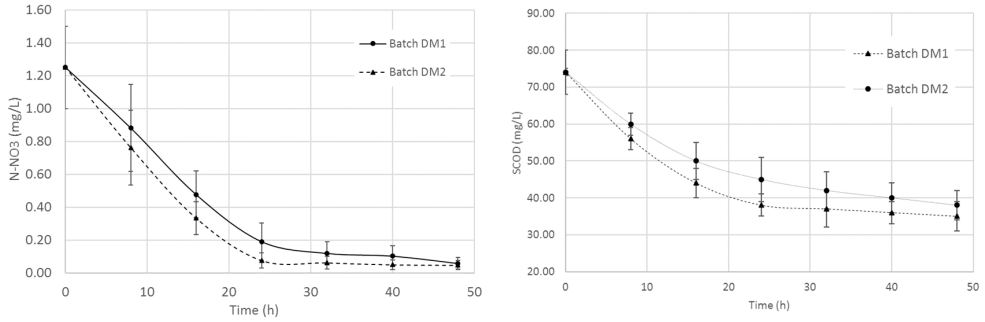
Figure 4. Dissolved oxygen with time during OUR test for *R. capsulatus* grown in aerobic-dark (AE-DK) and anaerobic-light (AN-LT) conditions.

Since the kinetic parameters of nitrification by respirometric measurements (stoichiometry of oxygen depletion due to ammonium oxidation) are well established [34], an increase of the OUR could be related to nitrification due to *R. capsulatus*.

R. capsulatus has confirmed to be very versatile and able to rapidly adapt its metabolism and its enzymatic legacy to changes in environmental conditions. Moreover, it was also clear that adding *R. capsulatus* to wastewater as an external inoculum could lay the ground for process optimization in both aerobic and anoxic environments.

3.3. Batch Experiments on Mixed Liquor from Municipal Wastewater Biological Treatment Plant

Wastewater treatment for the removal of organic carbon (COD) and nitrogen [35,36] is usually achieved through several steps in the treatment process such as primary settling, bioadsorption, and biodegradation, followed by a secondary settling step to remove the sludge flocs [37]. In particular, nitrogen can be transformed and removed by biologically mediated nitrification (aerobic process) and denitrification (anoxic process). Nitrification is a process which converts ammonia to nitrites and then to nitrates, while denitrification results in the transformation of nitrates and nitrites into nitrogen gas [38]. Figure 5 shows the results of the batches of denitrification processes on mixed liquor (batches DM1 and DM2) by following N-NO_3, and the SCOD concentration over time. It is worth noting that in both cases of inoculum of *R. capsulatus*, the efficiency of the nitrate (Figure 5a) and SCOD removal (Figure 5b) did not show significant differences. When *R. capsulatus* was added, in 25–30 h the nitrate was almost completely removed (>95%) from the batches. The corresponding SCOD moved up to 69% of the removal. No significant difference could be noted when inoculating mixed liquor with AE-DK or AN-LT biomass. *R. capsulatus* was confirmed to be a versatile strain, capable of adapting its metabolism to variable environmental conditions.



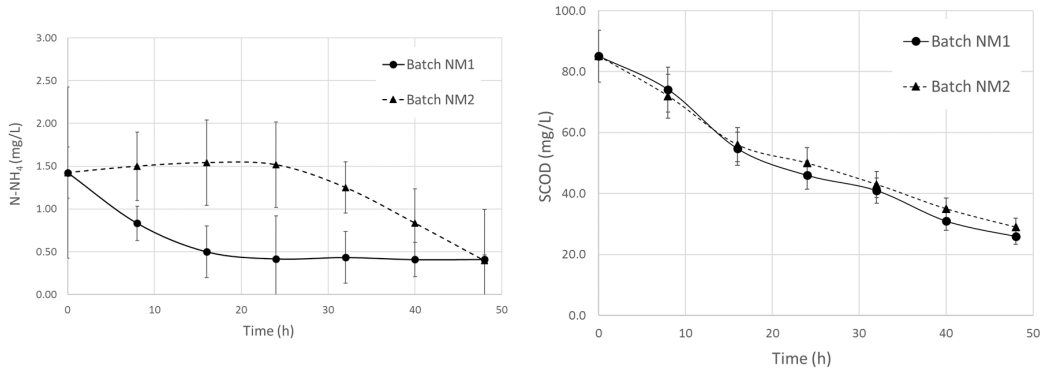
(**a**) (**b**)

Figure 5. Effect of denitrification of mixed liquor inoculated with *R. capsulatus* grown in aerobic-dark (batch DM1) and anaerobic-light (batch DM2) on nitrate (**a**) and SCOD (**b**) concentration.

Under aerobic-dark conditions, aerobic respiration is the main catabolism pathway for organic matter degradation through the glycolysis, tricarboxylic acid cycle and electron transport chain process, whereas under anaerobic-light conditions, material catabolism pathways are glycolysis and the following fermentation, or anaerobic respiration [39]. In the first case, oxygen is the final electron acceptor and the initial matrixes can be completely mineralized to CO_2 (oxidation tank conditions). In the latter, the final electron acceptors could be small products (fermentation) or nitrate (anaerobic respiration). In the nitrification tank (aerobic conditions, batches NM1 and NM2), N-NH_3 was reduced by 70% in 50 h when both AE-DK and AN-LT biomass were added.

Under highly aerobic conditions, biomass grown in AN-LT needed a long lag phase to express enzymatic activities for supporting the rearrangement of the metabolic pathways. However, after 24 h the rate of N-NH_3 removal from the mixed liquor reached almost the same amount for both inocula, corresponding to a SCOD decrease of 70% (Figure 6). The capacity of *R. capsulatus* to assimilate ammonia has been already established [40,41], indicating that it could be heterotrophically consumed for cell growth. Otherwise, the increase of the nitrate concentration could reflect some kind of nitrifying behavior as reported in the literature for other *Rhodobacter* spp. isolated from the soil [42]. Further investigations are necessary to evaluate if heterotrophic nitrification [43] could be one of the well-known metabolisms of *R. capsulatus*.

These results indicate that inoculating mixed liquor with *R. capsulatus* promotes the efficiency of both nitrification and denitrification conditions. Nevertheless, it is quite surprising that the conditions in which the biomass had grown seemed not to have an overall great influence on the process's performances, except for the occurrence of a lag phase after the inoculum due to the unavoidable necessity of rearranging the enzymatic legacy in order to adapt to the different environmental conditions.



(**a**) (**b**)

Figure 6. *Cont.*

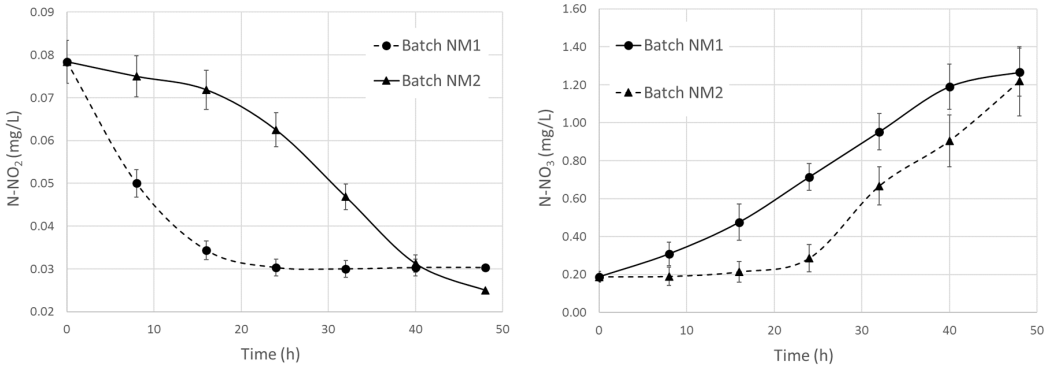


Figure 6. Effect of nitrification of municipal mixed liquor inoculated with *R. capsulatus* grown in aerobic-dark (batch NM1) and anaerobic-light (batch NM2) on ammonia (**a**) and SCOD (**b**) nitrate (**c**) and nitrite (**d**) concentration.

At this point, the discriminant factor that may suggest the use of AE-DK or AN-LT conditions is only the cost of production, in terms of substrate supply and equipment. To give a first and approximate economic evaluation between the two growth conditions tested, some preliminary calculations have been carried out. In particular, the 19-component MR medium for anaerobic-light growth had an average cost of 1.6 €/L while the aerobic-dark medium, which contained only peptone and meat extract, had a cost of about 0.9 €/L. Moreover, both components could be quite easily replaced with low-cost agro-food byproducts as sources of nutrients, further reducing the average price. For industrial applications, that means large volumes and big plants. For anaerobic-light–grown biomass, higher costs have to be unavoidably accounted for due to energy supply for light and the equipment for maintaining an anaerobic environment. Nevertheless, it is worth noting that in anaerobic conditions, the cell concentration obtained was an order of magnitude larger than for AE-DK, which contributes to considerable money savings in terms of the quantity of biomass to be produced.

4. Conclusions

The use of *R. capsulatus* as an external inoculum for biological processes in municipal wastewater treatments seems to be promising for both denitrification and nitrification. Moreover, in both cases, comparing the cost-effectiveness and the performance, biomass grown in aerobic-dark conditions seems to be more convenient for large-scale industrial applications, even though the biomass yield is of an order of magnitude less than in anaerobic-light conditions.

Author Contributions: Stefania Costa, Saverio Ganzerli and Irene Rugiero performed all the experiments and carried out all the analytical assays, also giving a great contribution to the discussion. Simone Pellizzari conceived and designed the experiments, together with Elena Tamburini, who wrote the manuscript. As supervisor of the research group, Paola Pedrini defined the general research statement.

Conflicts of Interest: The authors declare no conflict of interest.

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