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A N I N T E R N A T I O N A L J O U R N A L

Chemical Composition of *Rhodocyclus gelatinosus* Biomass Produced in Poultry Slaughterhouse Wastewater

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ABSTRACT

Rhodocyclus gelatinosus *R_1 grew photoautotrophically in poultry slaughterhouse wastewater inside glass columns (90x670 mm) during 7 days at 31 ± 4°C, under anaerobiosis and lightness supplied by daylight plus 3 (100W) incandescent and 4 (40W) fluorescent lamps. The culture was centrifuged (4,500xg/20 min) and lyophilized to originate a bacterial biomass with 7.1% moisture content. Chemical composition investigation showed 67.6% crude protein, 27.6% total carbohydrate, 0.6% lipids and 4.2% ash (dry weight). Amino acid composition of the biomass was similar to others described in the literature for* Rhodocyclus gelatinosus *and for other photosynthetic bacteria. Effluent COD removal after cultivation and elimination of the biomass was around 90%. The valuable chemical composition of* Rhodocyclus gelatinosus *R_1 biomass and the high content in essential amino acids signs for the potential use of the product in poultry feed.*

Key words: *Rhodocyclus gelatinosus*, biomass, poultry slaughterhouse wastewater, chemical composition, amino acid composition, COD removal

INTRODUCTION

Biomass production for use as protein source in human foods or animal feeds comprises the production of dried cells of different microorganisms (algae, actinomycetes, bacteria, yeasts, molds and higher funghi) grown in large-scale culture systems. This activity represents a promising application of biotechnology, which is even more successful when associated to the utilization of sewage or industrial wastes as substrate (Litchfield, 1983). Anoxygenic phototrophic bacteria, especially Purple Non-Sulfur Bacteria (PNBS) are widely distributed in nature (soil, water and wastes) and have special importance because of their potential role in the degradation of industrial pollutants (Sasikala et al., 1995). Photosynthetic bacteria grown in such substrates are known to produce biomass rich in protein with good contents of essential amino acids, besides carbohydrates, lipids, minerals, vitamins and carotenoids, what suggests the potential use of the product in animal feed (Prasertsan et al., 1993[a,b]). *Rhodocyclus gelatinosus*, a PNSB, is of common occurrence in many industrial effluents where it grows well photoautotrophically because of the ability to hydrolyze starch and gelatin due to peculiar hydrolyzing enzymes it produces. It also produces carotenoid pigments of the spirilloxanthin

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alternative series, which confers red color to its cultures (Trüper and Imhoff, 1992; Brock et al., 1994; Holt et al., 1994) and may find application in animal feed due to the possibility of intensifying animal tissue color (Kobayashi and Kurata, 1978; Noparatnaraporn and Nagai, 1986). This work investigated the chemical composition of *Rhodocyclus gelatinosus* biomass produced in poultry slaughterhouse wastewater aiming at a further use in the supplementation of broiler and laying hens rations.

MATERIALS AND METHODS

Substrate: Poultry slaughterhouse wastewater was filtered thrice (once through a metal sieve and twice through paper filter Whatman™ # 3), steam heated for 40 minutes and cooled to 25°C. This operation was repeated for three consecutive days.

Microbial culture: *R. gelatinosus* R_1 was isolated from poultry slaughterhouse wastewater as described previously (Ponsano, 2000).

Culture medium: Pfennig's medium (Pfennig, 1974) containing (g/l) KH_2PO_4 0.5; $MgSO_4.7H_2O$ 0.4; NaCl 0.4; NH_4Cl 0.4; $CaCl_2.2H_2O$ 0.05; organic compound 1; yeast extract 0.2; ferric citrate 0.005; trace elements solution 10 ml ($FeSO_4.7H_2O$ 200 mg; $ZnSO_4.7H_2O$ 10 mg; $MnCl_2.4H_2O$ 3 mg; H_3BO_3 30 mg; $CoCl_2.6H_2O$ 20 mg; $CuCl_2.2H_2O$ 1 mg; $NiCl_2.6H_2O$ 2 mg; $Na_2MoO_4.2H_2O$ 3 mg) was used (pH 7.0). It was autoclaved at 121°C for 15 minutes.

Vitamin solutions: Biotin 0.0015% sol.: biotin 1.5 mg; distilled water 100 ml. Membrane filtration on Millipore™ 0.22 μm. Thiamine 0.005% sol.: thiamine-HCl 5 mg; distilled water 100 ml. Membrane filtration on Millipore™ 0.22 μm.

Inoculum production: Loops of *R. gelatinosus* R_1 maintained in semi-solid agar (Pfennig's medium plus 1.2% bacteriological agar) was transferred to screw-cap test tubes (15x180mm), which were completely filled with Pfennig's medium enriched with 1 ml/l of each vitamin solution (both added after autoclaving). Tubes were tightly closed to keep anaerobiosis atmosphere and incubated for 7 days in an oven at 32 ± 2°C and 1400 ± 200 lux, generated by incandescent (40W) and fluorescent (15W) lamps. The contents of these tubes were transferred to 500 ml glass cylinders (10% v/v), which were filled with Pfennig vitamin-enriched medium and closed with glass stoppers. Incubation conditions were the same as described above, until the cultures reach absorbance of 0.3 at 600 nm (7 to 10 days), read in a Femto model 432 spectrophotometer, using Pfennig's medium as the blank.

Biomass production: Contents of the glass cylinders were slowly transferred (10% v/v) to glass columns (90x670 mm), which were filled with substrate and covered with rubber stoppers for anaerobiosis environment. Incubation was carried at 31 ± 4°C and lightness was supplied by the daylight plus 3 (100W) incandescent and 4 (40W) fluorescent lamps. After 7 days, these cultures were centrifuged at 4,500 x g for 20 minutes (Beckman model J 6M) at 0°C and the slime was lyophilized (MLW model LGA 05), resulting in *R. gelatinosus* R_1 biomass.

***R. gelatinosus* R_1 biomass composition:** Moisture, crude protein, lipid and ash contents of the biomass were determined according to Brasil (1998). Total carbohydrate was found by difference. Amino acid composition was determined by HPLC after cell hydrolysis with HCl 6N at 105°C for 22 hours with post-column reactor, ninhydrin as color reagent and UV detector at 520 nm for the readings. Analyzes were performed in triplicates.

Process productivity: Productivity was calculated from total volume of substrate utilized and total grams of biomass produced in the process, considering 7 days of cultivation (Aiba et al., 1971).

Chemical Oxygen Demand: Chromosulfuric acid oxidation method (COD cell test – Merck™) was used for COD determination on the effluent before cultivation and after cultivation and biomass elimination.

RESULTS AND DISCUSSION

Lyophilized *R. gelatinosus* R_1 biomass showed 7.1% moisture and chemical composition showed in Table 1. The high content of protein found to *R. gelatinosus* R_1 biomass is a typical characteristic for photosynthetic bacteria biomass, signing for a possible use of the product in animal rations formulation.

Table 1 - Composition of *R. gelatinosus* R_1 biomass

Nutrient	%	
	Dry weight	Wet weight
Moisture	---	7.1
Crude protein	67.6	62.8
Total carbohydrate	27.6	25.6
Lipid	0.6	0.5
Ash	4.2	4.0

Other authors have investigated the chemical composition of many photosynthetic bacteria biomass grown in different substrates. Balloni et al. (1986) used an association of photosynthetic bacteria (*R. gelatinosus* among others) in pig farm waste to produce biomass with 7.5% moisture, 50.6% protein, 12% carbohydrates, 22.5% lipids and 15.5% ash (d.w.). In another experiment, Balloni et al. (1987) cultivated *R. pseudomonas* and *R. fulvum* in sugar refinery wastewater and found a biomass with 58% protein, 16.7% carbohydrates, 13.5% lipids and 12.8% ash (d.w.). Sasaki et al. (1981) cultivated two strains of *R. gelatinosus* in a *miso*-like effluent medium and found 62 and 63% protein in the bacteria biomass. Noparatnaraporn and Nagai (1986) cultivated *R. sphaeroides* P47 in dehydrated medium from pineapple peel waste and obtained a biomass with 66.6% protein. Kobayashi and Kurata (1978) cultivated *Rhodopseudomonas capsulata* in a synthetic medium and found the following composition (dry weight) for biomass: 60.9% crude protein, 9.9% lipids, 20.8% soluble carbohydrates, 2.9% crude fiber and 5.3% ash. These authors also tested the effect of mixing biomass into laying hens feed and found that the egg-laying rate tended to increase with the addition of photosynthetic bacterial cells. They also observed improvements in yolk color and carotenoid contents depending on the quantity of added bacterial cells, suggesting that the biomass carotenoids could be well absorbed and transferred into yolk.

Table 2 shows amino acid composition of *R. gelatinosus* R_1 and other photosynthetic bacteria biomass. Lysine, methionine, glycine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan and valine were present in good proportions in *R. gelatinosus* R_1 biomass. According to Andriguetto et al. (1988), these are considered essential amino acids to lead to a maximum yield in poultry nutrition. *R. gelatinosus* R_1 biomass amino acid composition was very similar to the findings of other authors for most amino acids, except to methionine and phenylalanine, which were in slightly lower concentrations (Kobayashi and Kurata; 1978; Sasaki et al., 1981; Noparatnaraporn and Nagai, 1986). The essential amino acids contents of *R. gelatinosus* R_1 biomass may be considered similar to those of egg and soybean (Sasaki and Nagai, 1979), algae (Prasertsan et al., 1993[a]), yeast, animal and plant proteins (Noparatnaraporn and Nagai, 1986).

Some researchers have investigated photosynthetic bacteria biomass production in industrial effluents, such as sugar refinery wastewater (Balloni et al., 1987), *miso* factory wastewater (Sasaki et al., 1981) and pineapple peel waste (Noparatnaraporn and Nagai, 1986). While serving as a substrate, the pollutant load of the waste is reduced, as it happens in a sewage treatment. This may represent an advantageous practice to food industries because substrate cost is low, cost with installation of wastewater purifying equipment may be minimized, contamination control in process is easy, organisms utilized are not pathogenic and have high nutritional value (Kobayashi and Kurata, 1978). Prasertsan et al. (1993[a,b]) isolated and identified *R. gelatinosus* from seafood processing wastewater and cultivated the organism in the same substrate to investigate growth parameters and chemical oxygen demand (COD) removal. Authors found a biomass with 50% protein and 86% COD removal. Cultivation conditions described in this study led to an average 90% COD removal for poultry slaughterhouse wastewater. This process may be feasible and advantageous to the poultry industry once little expenses with wastewater treatment are required. Simple operations like filtration and decantation are enough to make effluent

appropriate for *R. gelatinosus* R_1 biomass production and sterility is not necessary because *R. gelatinosus* R_1 predominates over other competing specia in this substrate.

Process productivity was around 0.072 g/l.day, since 250 g of biomass were produced from 500 l of poultry slaughterhouse wastewater during seven days of cultivation. Productivity is typically lower for phototrophic bacteria biomass production than it is for heterotrophic organisms because cell densities achieved are low (Kobayashi and Kurata, 1978; Litchfiled, 1983). This is in agreement with other authors' works carried out with different photosynthetic bacteria (Sasaki and Nagai, 1979, Sasaki et al., 1981; Noparatnaraporn et al., 1983; Balloni et al., 1986; Noparatnaraporn and Nagai, 1986; Balloni et al., 1987; Prasertsan et al., 1993[a,b]). Cultivation of *Rhodocyclus gelatinosus* R_1 in poultry slaughterhouse wastewater was feasible due to the simple incubation conditions

Table 2 - Amino acid composition of *Rhodocyclus gelatinosus* R_1 biomass and other photosynthetic bacteria

	% (dry weight)				
Amino acid	*R. gelatinosus* R_1	*R. capsulata* (Kobayashi and Kurata, 1978)	*R. gelatinosus* (Sasaki et al., 1981)	*R. gelatinosus* A1 (Sasaki et al., 1981)	*R. sphaeroides P47* (Noparatnaraporn and Nagai, 1986)
aspartic acid	5.74	4.56	NA	NA	NA
tyrosine	2.90	1.71	NA	NA	NA
serine	2.63	1.68	NA	NA	NA
glutamic acid	6.85	5.34	NA	NA	NA
proline	3.27	2.80	NA	NA	NA
glycine	4.18	2.41	NA	NA	NA
alanine	6.98	4.65	NA	NA	NA
tryptophan	1.74	1.09	NA	NA	NA
cysteine	0.59	NA	NA	NA	NA
valine	4.56	3.51	3.42	3.75	2.68
methionine	1.40	1.58	1.89	1.71	1.47
isoleucine	3.18	2.64	2.73	2.96	1.78
leucine	6.80	4.50	5.41	5.28	3.90
threonine	3.52	2.70	1.99	1.93	2.87
phenylalanine	3.03	2.60	3.10	3.05	2.36
lysine	3.61	2.86	3.12	3.41	2.57
histidine	1.83	1.25	1.13	1.01	0.96

NA. not analyzed

required and profitable due to the valuable nutrient composition of the biomass produced. Moreover, the process led to a substantial COD removal in poultry slaughterhouse wastewater signing for an alternative waste treatment method. These characteristics make *Rhodocyclus gelatinosus* R_1 an interesting microorganism for the production of biomass aiming at the supplementation of broiler and laying hen nutrition.

RESUMO

Rhodocyclus gelatinosus R_1 foi cultivado fotoautotroficamente em águas residuais de abatedouro de aves dentro de colunas de vidro (90x670 mm) durante sete dias a 31 ± 4°C, sob anaerobiose e intensidade luminosa fornecida pela luz do dia e por três lâmpadas incandescentes (100W) mais quatro lâmpadas fluorescentes (40W). O cultivo obtido foi centrifugado (4.500xg/20 min) e liofilizado, originando a biomassa bacteriana, que continha 7,1% de umidade. A determinação da composição centesimal indicou (base seca) 67,6% de proteína bruta, 27,6% de carboidratos totais, 0,6% de

lipídeos e 4,2% de cinzas. A composição em aminoácidos da fração protéica da biomassa mostrou-se semelhante à relatada na literatura para *Rhodocyclus gelatinosus* e para outras bactérias fotossintetizantes. A redução na DQO do efluente após o cultivo e a retirada da biomassa foi da ordem de 90%. A valiosa composição química da biomassa de *Rhodocyclus gelatinosus* R_1 e o alto conteúdo em aminoácidos essenciais indicam o uso potencial desse produto na suplementação de ração para aves.

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