Influence of the Addition of Riboflavin in Culture Medium on Delivering Biomass Using Yeast Strains of *Saccharomyces Carlsbengensis*

Cornelia Nicoară

School Group „Stefan Manciulea", Blaj, 515400, str. Piata 1848, nr.3, Romania

Abstract
Yeasts requirements for growth factors should be considered both in terms of ability to summarize the simple average and the dependence on external supplies. Vitamins are components of coenzymes or enzymes prosthetic groups and thus they are growth factors for yeast. The study concerns about the influence of the addition of riboflavin in culture medium in different quantities, the accumulation of yeast biomass under the action of yeast strains of beer. The process of cultivation has been made for 24 hours at a temperature of 22^0C. The addition of riboflavin in culture medium of yeast biomass increased in each strain of yeast compared with the witness - the sample without added riboflavin. Biomass obtained by follow this procedure could be used to create new food products with high ration nutritional value.

Keywords: biomass, environment, riboflavin, *Saccharomyces carlsbergensis.*

1. Introduction

Many research aimed possibility of increasing the yield of production of yeast for beer, both under the influence of physical and chemical factors. The study of these factors lead to necessity and promote new cultivation methods aimed to increasing fermentative activity and extension of storage.The process for obtaining biomass of yeast is characterized by complex interdependencies between intrinsic factors (composition of the cultivation environment, rate of substrate supply, pH and rH of the environment etc.) and extrinsic factors (temperature, breathing rate, aeration rate, specific growth rate etc.) [1, 2]. All these factors complete a whole unit that contributes to growing and metabolism of active cells in the optimal cultivation of yeasts. Vitamins stimulate the growth of all microorganisms because the biosynthetic cargo are decrease in yeast cells. [2]. In fact, they need this process.

* Corresponding author: Nicoara Cornelia, 0721817928, cornelia_nicoara@yahoo.com

The study of biomass accumulation and determination of optimal conditions for cultivation of yeasts in bioreactors under aerobic conditions was performed in the microbiology laboratory of the Center for Biotechnology at the Faculty of Agricultural and Food Industry, "Lucian Blaga" led by Mrs. Prof. Biol. Letitia Oprean.

2. Materials and methods

Culture media:
Malt mash (MM) containing malt extract 15 g / l, peptone 1 g / l, maltose 12.5 g / l, dextrin 2.5 g / l, potassium phosphate 1 g / l, ammonium chloride 1 g / l, pH 4.8 (Scharlau Chemie SA, Spain).
Synthetic Environment (MS),pH 3.8 composed of:
- Phosphate (KH_2PO_4) 5 g / l
- Ammonium sulphate (NH_4) $2SO_4$ 2 g / l
- Magnesium sulphate ($MgSO_4$) x7 H_2O 0.4 g / l
- Yeast extract 1 g / l
- Glucose 50 g / l

Vitamins: riboflavin (B_2) in proportions: 0.01 mg/l, 0.05 mg/l and 0.10 mg/l.
Yeast cultures:

In the study were used: three strains of brewer's yeast (denoted by SCL3, SCL4, SCL5) which were compared with a control strain considered: *Saccharomyces carlsbergensis* SCL W 34, coming from Freiburg, Germany.

Equipment used in determinations:
Laboratory bioreactor 5 l (4 l useful volume) of type Biostat A, B, Braun Biotech International, equipped with a computerized monitoring, control and registration, with sensors for temperature, dissolved oxygen, oxygen evolved, carbon dioxide , and optical sensor recording of biomass. The transformation of values read by optical sensor units biomass (g/l) is done with specific calibration curve of beer yeasts.

Methods:
Production of biomass by yeast is released by known phenomenon as the Pasteur Effect, like inhibition of fermentation by respiration. For this fact, yeast is grown under conditions of intense aeration, the concentration of sugar in the environment is kept low to avoid formation of alcohol, for obtaining biomass [3].

Culture media were enriched with riboflavin and the bioreactor autoclaved at a temperature of 120^0C for 30 minutes. The volume used was 4 liters. After cooling, yeast is introduced in quantities of 20 grams (wet biomass), the results is referring to this value also. The volume of air blow was set at 100 l/h, results an aerobic fermentation.
Fermentation control was selected from computer software, including all parameters. The results have been selected for each fermentations in part, reading the sensor value is converted to biomass using calibration curves in g/l.

3. Results and discussion

The amount of biomass accumulated in the two cultures of media, malt mash (MM) and synthetic medium (SM), which were added different quantities of riboflavin was measured at 4 hours, 8 hours, 12 hours and 24 hours. Fermentation was performed at 22^0C, and the amounts of biomass accumulated in the two culture media are presented in Table 1 and Table 2.

Table 1. Accumulation of biomass under the influence of the addition of riboflavin in culture medium, malt mash.

Nr. crt.	Strain studied	Temperature (°C)	Concentration of riboflavin added (mg / l)	The amount of biomass accumulated, g / l			
				4 hours	8 hours	12 hours	24 hours
1	SCL W 34	22	**blank assay**	47,21	49,23	37,65	33,58
			0,01	48,77	45,35	39,26	39,12
			0,05	51,49	46,84	43,00	39,41
			0,10	49,09	43,36	40,93	39,81
2	SCL 3	22	**blank assay**	47,64	49,55	37,94	33,42
			0,01	48,74	45,52	39,93	39,04
			0,05	51,84	46,94	43,53	39,86
			0,10	49,05	43,93	40,04	39,91
3	SCL 4	22	**blank assay**	47,55	49,93	37,93	33,33
			0,01	48,93	45,05	39,93	39,55
			0,05	51,93	46,95	43,88	39,92
			0,10	49,83	43,41	40,11	39,86
4	SCL 5	22	**blank assay**	47,38	49,04	37,95	33,11
			0,01	48,58	45,55	39,64	39,53
			0,05	51,95	46,95	43,84	39,99
			0,10	49,40	43,32	40,00	39,04

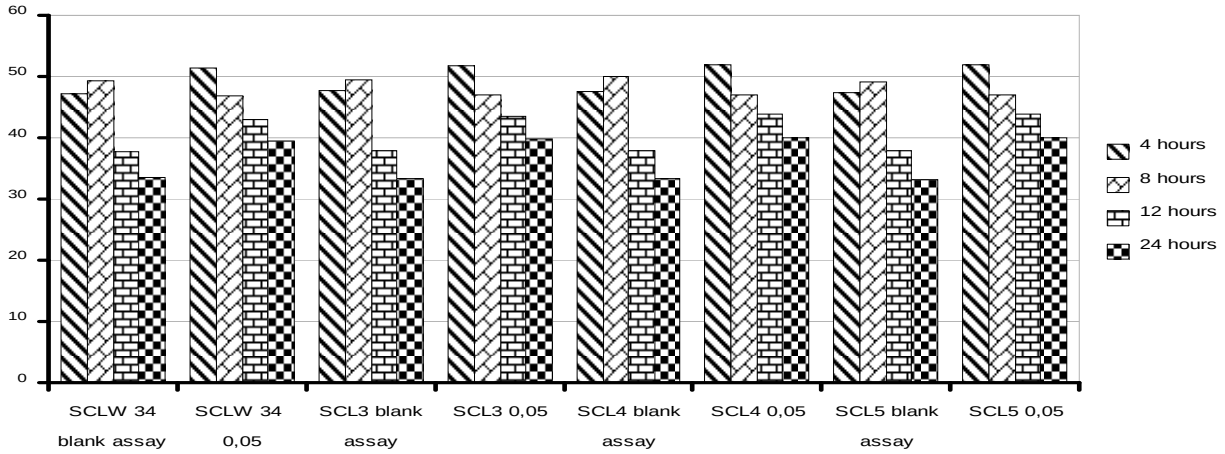

Figure 1. Accumulation of biomass under the influence of the addition of riboflavin in culture medium malt mash

Riboflavin has a very important and multiple role. It enters into the constitution of the FMN dehydrogenase and FAD, helping in reduction-oxidation reactions. Riboflavin is important in the oxidation of amino acids, the synthesis and fatty acid oxidation and glucose oxidation - the three processes involved in energy production.

The main results after an added incentive quantity of riboflavin, 0.05 mg per liter quantity of biomass increased viral SCL5, SCL4 and SCL3 versus blank sample (without riboflavin).

Table 2. Accumulation of biomass under the influence of the addition of riboflavin in synthetic medium culture

Nr. crt.	**Strain studied**	**Temperature (°C)**	**Concentration of riboflavin added (mg / l)**	**The amount of biomass accumulated, g / l**			
				4 hours	**8 hours**	**12 hours**	**24 hours**
1	SCL W 34	22	**blank assay**	47.39	49.08	37.40	33.17
			0.01	48.41	45.41	39.73	39.05
			0.05	51.97	46.11	43.78	39.15
			0.10	49.10	43.65	40.64	39.14
2	SCL 3	22	**blank assay**	47.45	49.99	37.45	33.41
			0.01	48.66	45.89	39.75	39.09
			0.05	51.41	46.77	43.65	39.44
			0.10	49.74	43.666	40.23	39.65
3	SCL 4	22	**blank assay**	47.45	49.56	37.11	33.41
			0.01	48.33	45.52	39.98	39.32
			0.05	51.41	46.45	43.45	39.44
			0.10	49.65	43.77	40.14	39.41
4	SCL 5	22	**blank assay**	47.16	49.45	37.96	33.74
			0.01	48.15	45.74	39.2	39.01
			0.05	51.14	46.98	43.35	39.66
			0.10	49.24	43.62	40.141	39.74

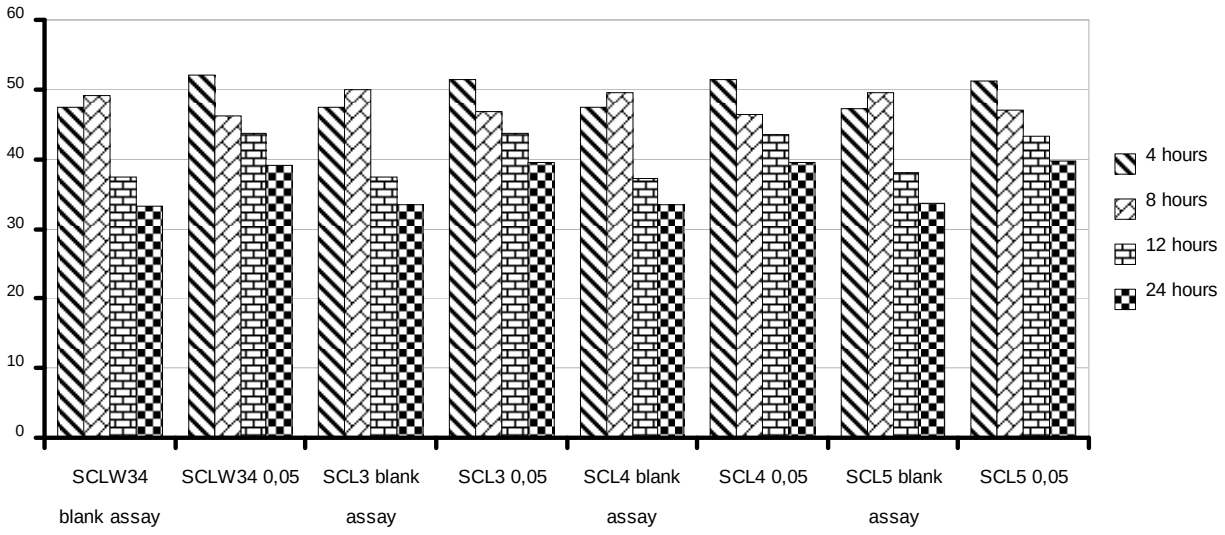

Figure 2. Accumulation of biomass under the influence of the addition of riboflavin in culture synthetic medium

Follows the added incentive quantities of riboflavin, 0.05 mg per liter, the amount of biomass has increased on the strain of yeast in the following order SCL5, SCL4 and SCL3 compared with blank assay.

4. Conclusions

Analyzing the results obtained by cultivating strains of *Saccharomyces carlsbergensis* in different culture media with different additions of riboflavin found that in comparation with blank assay, all other samples had increased biomass.

It can be seen in the two tables that the first 4 hours of fermentation, yeast biomass has increased in each strain, which shows that during this period has been a very active multiplication of yeasts. The maximum amount of biomass accumulated in this case has been registered on SCL 4.

Towards the end of fermentation the amount of biomass is approximately constant multiplication and capacity of multiplication is slowed.

Since the synthetic culture medium contains glucose, the amounts of yeast biomass obtained in this case are slightly lower than for cultivation of yeast strains on culture medium malt mash.

Adding riboflavin in the culture medium in a moderate quantity, the process of fermentation is being stimulated having a pozitive effect on the acumulation of biomass. This biomass can be used as raw material for different food products.

5. References

1. Dumitru, I.F., A. Vamanu, O. Popa, Drojdiile. Biotehnologii clasice si moderne, Ed. Ars Docendi, Bucuresti, 2002

2. Vamanu, A., G., Campeanu, S., Campeanu, E., Vamanu, Carmen Campeanu, Biotehnologii microbiene, *vol. I*, Ed. Ars Docendi, Bucuresti, 2003

3. Oprean Letitia, Drojdii industriale, Ed. Universitatii „Lucian Blaga", Sibiu, 2002

4. Banu C., Tratat de stiinta de tehnologia maltului si a berii, *vol. II*, Ed. AGIR, Bucuresti, 2001

5. Oprean Letitia, Mironescu Monica, Microbiologie. Lucrari Practice, Ed. Universitatii „Lucian Blaga", Sibiu, 2000.