Bioprocess design to produce resveratrol

Part 1: Preliminary

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General Analysis

WHAT is RESVERATROL? An antioxidant of plant origin. A biofenol naturally produced by such species as *Polygonum cuspidatum*, *Vitis vinifera* (grapes), *V. rotundifolia* and *Fallopia japonica* [1], when under attacks by pathogens as bacteria or fungi.

WHICH PROPERTIES make it SPECIAL? Resveratrol has recently become focus of numerous studies in plant physiology and medicine, and has emerged as a positive molecule in human health due to several potential benefits such as anticancer, anti-aging and anti-diabetes effects.

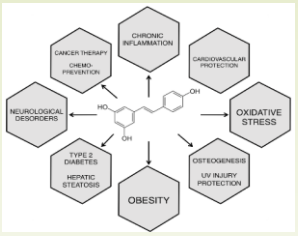

Figure 1. Potential benefits of resveratrol.

Market Outlook

- Special interest in USA and Europe.
- Growing demand in the Asia-Pacific region.
- 84% is sold as **dietary supplements**.
- **Lack of high quality** products due to the industrial chemical process usually used.

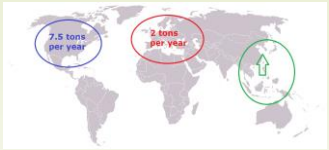

Figure 2. World map highlighting areas with higher resveratrol sales.

Plant Location

This facility's place would be in **Shanghai** (China) due to several reasons:

- Rare **raw materials** such as p-coumaric acid near.
- Most resveratrol producers are there. **Trained labor** near.
- Widespread **infraestructure network.**

Figure 3. Plant location in Shanghai (China).

Project Definition

Design of a production plant of resveratrol using SuperPro Designer v8.5 capable of covering the **global demand** based on a **biotechnological process**, in which, from p-coumaric acid, through a molecular transformation in a strain of *Escherichia coli* genetically modified and several chemical extractions to separate and purify the product, resveratrol is produced with a **purity greater than 99.5%**. The annual production reaches 12 tons of resveratrol sold as 500 mg **pills**.

General Process Block Diagram

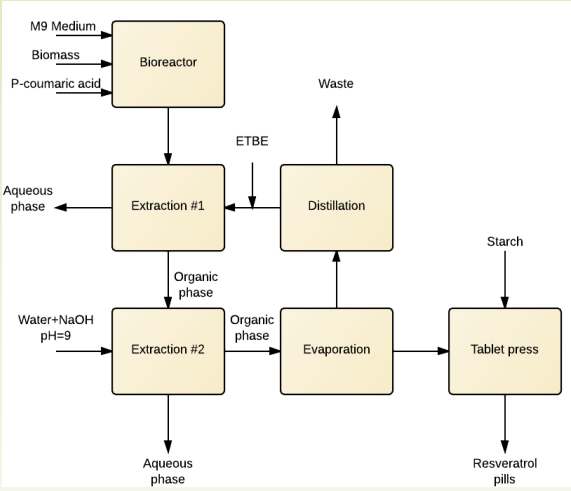

Figure 4. General process block diagram. ETBE: ethyl tert-butyl ether.

Process Control

Table 1. Variables controlled and their measurement methods. (*) Foaming is not controlled, but avoided.

Variable controlled	Measurement methods
Temperature, **pH** and **pO2**	Direct measurement with **sensors**
Cell concentration	Oxygen uptake rate (**OUR**) (**) and **optical density** at 600 nm
P-coumaric acid concentration	HPLC with a Synergi 4u max-RP80A column at 507 nm [3]
Resveratrol concentration	**Fluorescence** excitation spectrum at 310 nm [4] and emission spectrum at 400 nm and **HPLC** with a Synergi 4u max-RP80A column at 507 nm [3]
Foaming (*)	**Defoamers** added at the beginning of the process and foam controlled visually
Level control	Measurement of **differential pressure** in the top and the bottom of the vessel

Layout

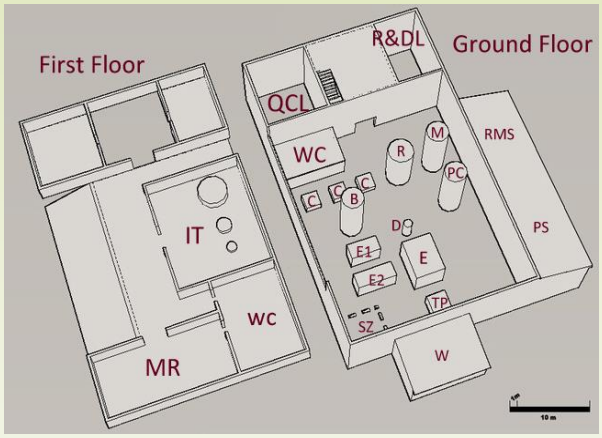

Figure 5. 3D structure of the production plant done with AutoCAD 2011. M: media tank, PC: p-coumaric acid tank, R: reactor, C: centrifuge, B: blending tank, E1: extractor #1, E2: extractor #2, E: evaporator, D: distillator, TP: tablet press, SZ: security zone, W: waste storage, RMS: raw materials storage, PS: product storage, QCL: quality control lab, R&DL: R&D lab, IT: inocule train, MR: meeting room, WC: water closet.

OUR measurement (**)

1. kLa measurement. Using "gas out – gas in" method, Oxygen would be removed from the media (without cells) by bubbling nitrogen. When oxygen concentration would reach zero again, oxygen would be fed using the same operational conditions. kLa can be calculated from the balance:

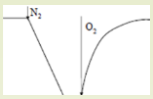

Figure 6. Oxygen profile using gas out – gas in method to calculate kLa.

$$\frac{dC}{dt} = k_L a \cdot (C^* - C) \rightarrow \ln(C^* - C(t)) = -k_L a \cdot t$$

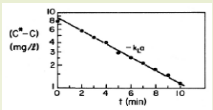

Figure 7. Linealization of integrated equation of gas out – gas in method.

2. OUR test. The aeration of the batch is interrupted and the resulting decrease in the oxygen concentration is observed as a function of time, that can be easy quantified with the following equation:

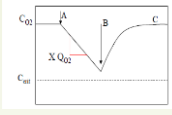

Figure 8. Representation of how OUR can be estimated.

$$C = \frac{-1}{k_L a} \cdot \left(\frac{dC}{dT} + XQ_{O2}\right) + C^*$$

References

1 - Mattivi, F., Reniero, F. & Korhammer, S. Isolation, Characterization, and Evolution in Red Wine Vinification of Resveratrol Monomers. *J. Agric. Food Chem.* **43,** 1820–1823 (1995).
2 - Donnez, D., Jeandet, P., Clément, C. & Courot, E. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends Biotechnol.* **27,** 706–13 (2009).
3 - Beekwilder, J. *et al.* Production of resveratrol in recombinant microorganisms. *Appl. Environ. Microbiol.* **72,** 5670–2 (2006).
4 - Piñeiro, Z., Palma, M. & Barroso, C. G. Determination of trans-resveratrol in grapes by pressurised liquid extraction and fast high-performance liquid chromatography. *J. Chromatogr. A* **1110,** 61–5 (2006).