

## Bioremediation of Olive Oil Mill Wastewaters by Fungal (*Trichoderma viride*, strain 8/90) Sequencing batch reactor

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Olive oil mill wastewaters (OOMWs) still represent an important environmental problem due to the several difficulties which has to be faced for their disposal. The performance of a lab-scale Sequencing Batch Reactor (SBR) for olive oil mill wastewater (OOMW) treatment was investigated. The reactor, preliminarily filled with suspended solids free OOWM was inoculated with *Trichoderma viride* (strain 8/90) fungal biomass. Influent and effluent dissolved organic carbon (DOC), total suspended solids (TSS) and polyphenols concentrations were monitored. The obtained results showed that the steady state removal of the organic carbon was about 66%, whereas phenolic compounds were reduced by about 50%.

### 1. Introduction

Olive oil is one of the most used food products in the world. Nevertheless oil is a typical product of the so called Mediterranean Region, which comprises Southern European (Spain, Italy, Greece), Northern African (Algeria, Morocco, Tunisia) and some Middle East (Turkey, Syria) countries, the olive oil market is very significant in all over the world, as reported in Figure 1. In 2005 the estimated world oil production (and consumption) was about  $2.5 \cdot 10^6$  tonnes (<http://www.unctad.org/>).

The New Zealand olive oil industry, even still small (2005 production was estimated to be about 112 tonnes, <http://www.olivesnz.org.nz>), is promisingly growing. An interesting factor for New Zealand new season's olive oils is that they are available on the market when the Northern hemisphere oils are already six months old. Even though New Zealand had no indigenous olive trees, a suitable cultivar of this species was introduced in the country with olives brought in during the nineteenth century.

Oil industry, however, raises concerns wherever it develops. The wastewater co-generated during the productive process – commonly named as Olive Oil Mill Wastewater, (OOMW) – is very polluting (Borja et al., 2006): it is characterized by a very high COD content, a low pH, a suspended solids fraction and features the presence of biorecalcitrant and inhibiting compounds – mainly polyphenols – which make traditional biological treatment processes scarcely effective. A typical OOMW characterization is reported in Table 1.

Table 1 – Typical OOMW (from Proietti and Nasini, 2006) and utilized wastewaters compositions.

| Parameter (unit)         | Typical OOMW Range | Utilized OOMW (soluble fraction) |
|--------------------------|--------------------|----------------------------------|
| pH                       | 4.3 ÷ 5.8          | 5.08                             |
| COD (g/l)                | 15 ÷ 390           | 136<br>(78)                      |
| Organic Carbon           | n/a                | 54<br>(34)                       |
| Organic Matter (g/l)     | 35 ÷ 150           | 45                               |
| Sugars (g/l)             | 5 ÷ 80             | 12*                              |
| Nitrogen Compounds (g/l) | 5 ÷ 24             | 1.1                              |
| Polyphenols (g/l)        | 3 ÷ 24             | 4.7*                             |
| Lipids (g/l)             | 1 ÷ 15             | n/a                              |
| P (mg/l)                 | 20 ÷ 1100          | 1740                             |

(\*) expressed as glucose equivalents  
(\*\*) expressed as gallic acid equivalents

So far, the disposal of OOMW is not a stringent environmental problem in New Zealand (contrary to the situation of the main producing countries, like Italy and Spain); however, as the New Zealand oil industry is promisingly growing, a preventive investigation for the set-up of a treatment technology may play an important role in the future. In this work, the results of a continuous OOMW-treating reactor are shown. The key point is that a selected fungal biomass (*Trichoderma viride*) has been deployed to inoculate the reactor; indeed, this fungal species has been demonstrated to be able to withstand critical conditions (low pH, high polyphenols concentration, high COD content) and to successfully operate the treatment process in both model (D'Urso et al., 2007a) and semi-model (D'Urso et al., 2007b) applications. Furthermore, other authors obtained interesting results utilizing OOMW spontaneous fungal flora for the treatment (Caffaz et al., 2007). Last, but not least, New Zealand has a remarkable track of smart uses of *Trichoderma* spp. biomass, such as crops biocontrol (Agrimm Technologies Limited, www.tricho.com).

## 2. Materials and Methods

### 2.1 Biomass

For this work, *Trichoderma viride* Pers:Fr. Isolate 8/90 has been used. It was kept as pure culture on Petri dishes and stored at 26° C.

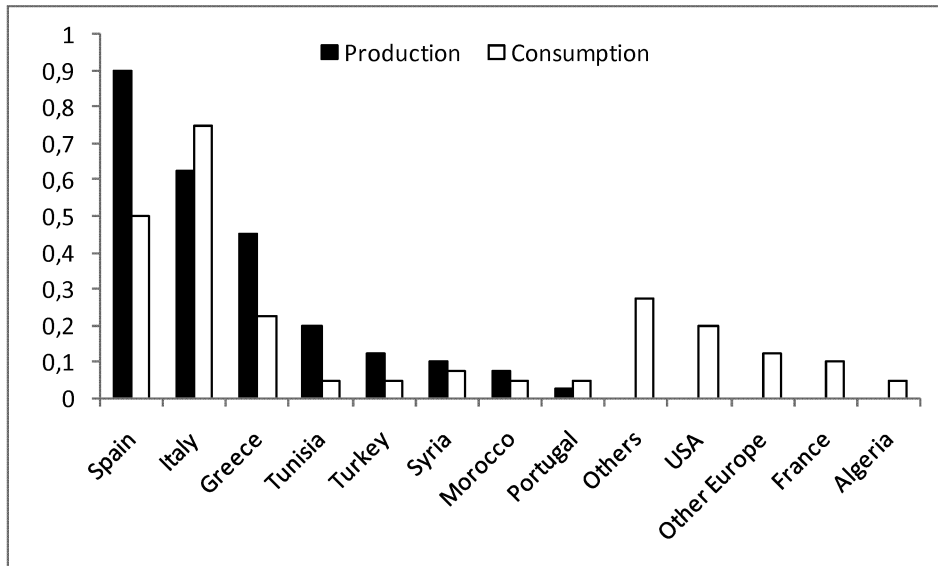


Figure 1 – Olive oil world production and consumption (10<sup>6</sup> tonnes), from <http://www.unctad.org>.

## 2.2 Wastewater

The OOMW was collected from “The Village Press” mill, located in Hawkes Bay, which is one of the most important regions for the production of extra virgin olive oil in New Zealand. After reception in the laboratory it was characterized, then stored at 4° C until use.

## 2.3 Reactor Management

The study was performed as a continuous treatment in a Sequencing Batch Reactor (SBR) system (volume = 2 l).

The SBR was fed with pre-treated OOMWs. They were centrifuged at 4000 rpm for 30 minutes and then filtered (75 micron); thus, a removal of about the 50% of the initial TOC level was achieved. This solid material has been shown from other authors to be eligible for an energy recovery by thermal conversion (Caffaz et al., 2007).

A biological treatment policy featuring equal Hydraulic Retention and Solid Retention times (HRT and SRT) of 5 days that is, without settling phase, was adopted for modelling purposes. The pH and temperature set points were fixed, respectively, at 3.5 (aim of this investigation was also identifying the biomass behaviour in extremely acidic conditions) and 25°C. The air flow rate was 3 vvm.

The reactor was managed as a batch one during the first three days, to allow for *T. viride* biomass growth: diluted (1:4) OOMW was introduced as growth medium. After 8 days, the centrifuged OOMW was continuously fed to the system.

The reactor was operated according to the following schedule:

- Load phase: 5 min;
- Reaction phase: 340 min;
- Discharge phase: 15 min.

### 3. Results and discussions

DOC, polyphenols concentration and suspended solids content, together with biomass concentration (VSS) and COD removal (these latter representing the quantities most descriptive of the quality of the devised treatment), were monitored during the whole duration of the experiment. The results of the performed treatment are reported in Figure 2.

During the start-up phase about 1300 mg<sub>DOC</sub>/l (i.e. 108 C-mmol) were removed and the biomass concentration rose to 950 mg<sub>VSS</sub>/l. Considering the general raw formula of microbial biomass (C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N), it means that 42 C-mmol of biomass were produced. Thus leading to an observed biomass yield of 0.39 (on a C-mmol base).

On the other hand, a polyphenols analysis showed that these compounds were not removed from the medium, likely because the biomass was not acclimatized and ready to use them for growth yet.

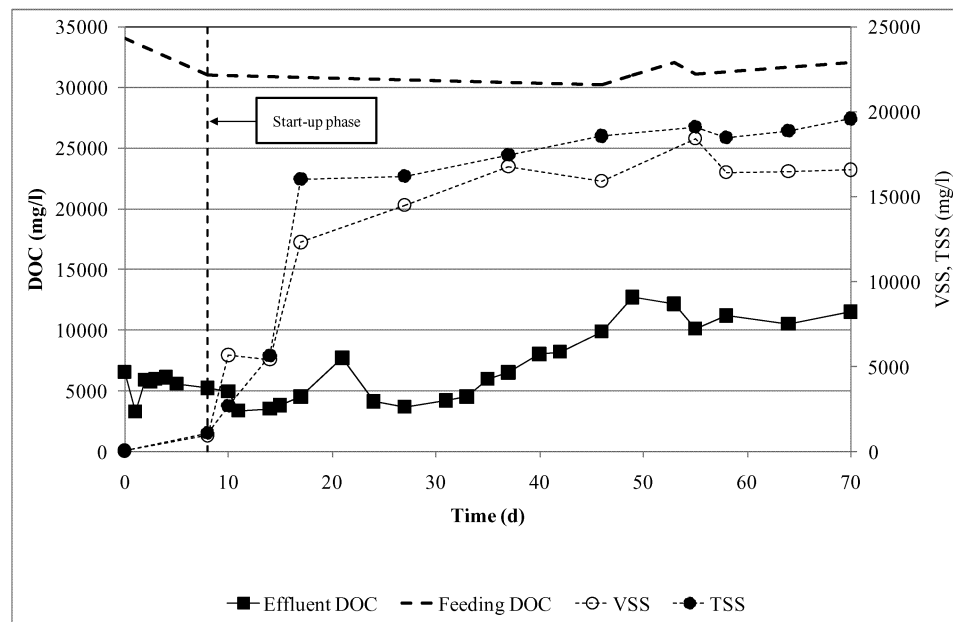


Figure 2 – Biomass profile and COD concentration in the outgoing effluent.

From  $t = 9$ d onwards, OOMWs were continuously fed to the reactor. Interestingly, DOC and polyphenols concentration followed two different behaviours.

DOC profile revealed that the biomass was immediately able to remove part of the fed soluble organic matter using it as a substrate for its growth. After about 45 days, the reactor reached a steady state, in term of DOC removal percentage and biomass concentration as well. Considering the average concentration of the feed equal to 30000 mg<sub>DOC</sub>/l and the (almost stationary) concentration in the outflow of about 11350 mg<sub>DOC</sub>/l, a 62% treatment efficiency can be calculated. At the steady state biomass concentration of 17000 mg<sub>VSS</sub>/l, this evaluates to an observed yield of 0.48 (on a C-mmol base), which is just a little bit higher with respect to that relevant to the start-up phase. The polyphenols profile, instead, revealed that these compounds were not

removed in both the batch start-up phase and in the first period of the experimentation, as can be depicted in Figure 3. However, from  $t = 17$  d onwards, the polyphenols level in the reactor effluent started to progressively decrease according to a linear profile.

A comparison between the actual time profile of the effluent polyphenol concentration and that calculated on the basis of their measured concentration in the feed, under the hypothesis of null removal efficiency (see Figure 3), highlighted that in the first 35 days no removal were occurring. Thereafter, the difference between the calculated values and the experimental ones became significant, thus indicating that the biomass had activated/adapted its metabolic machinery to degrade polyphenols as well.

Comparing these results with those in D'Urso et al. (2008), it is readily apparent that the development of the capability of degrading phenolics takes longer on OOWM than on a

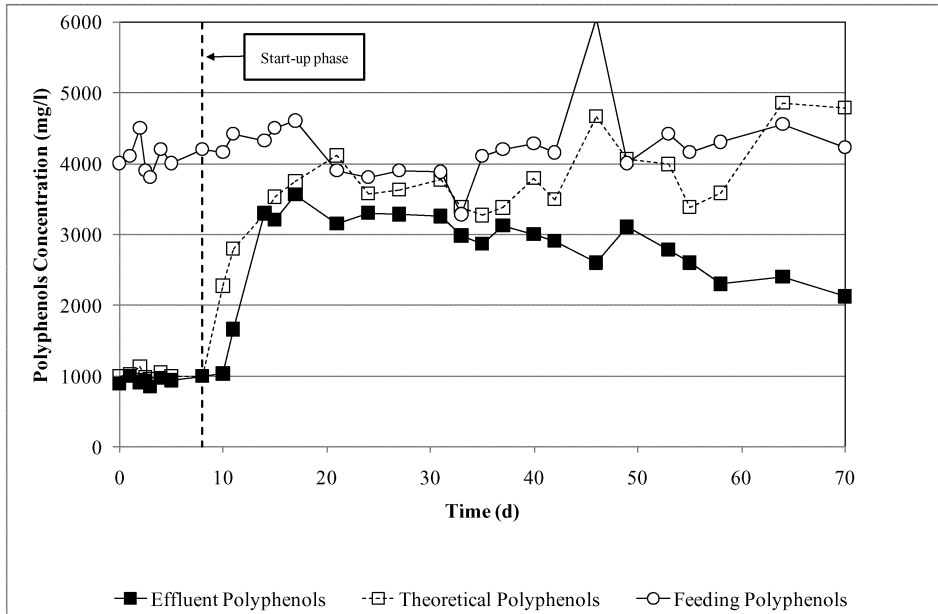


Figure 3 – Polyphenols concentration profiles. The theoretical values are calculated on the basis of the feeding concentration and considering an accumulation in the reactor (i.e., no removal).

synthetic medium including gallic acid. Most likely, this is due to the presence of complex structure phenolic compounds which take longer to degrade (or may never be degraded). Indeed, we showed earlier (D'Urso et al., 2007) that, while gallic acid is almost immediately metabolised, tannic acid was never removed from the broth.

#### 4. Conclusions

*T. viride* biomass showed the capability to grow on OOMW. DOC content removal reached a steady state and in the effluent a concentration of one third with respect to the influent level was found. Polyphenols removal, instead, was showing an increasing performance as the effluent concentration was going down with a linear-shape behavior.

A reasonable C-mol yield showed that the biomass was able to grow on the wastewater with no significant inhibition.

Although further studies should be done in order to transfer this technology to full-scale application (e.g. oxygen supply optimization) two practical conclusions can be immediately drawn: i. compounds of increased complexity might require time for the biomass to become able to treat them, during which the treated wastewater cannot be expected to meet the design specifications and ii. the biomass is able to grow in the medium with no inhibition at any time and therefore requires no particular care during the reactor start-up phase..

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