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## Background and Aim

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a compound that can be naturally found in bean pods of the tropical orchid *Vanilla planifolia*. Worldwide, there is an industrial demand for vanillin as a flavouring agent, fragrance ingredient or pharmaceutical precursor. Many methods were developed to produce vanillin: solvent extraction from cured *Vanilla* pods, a synthetic condensation process between guaiacol and glyoxylic acid with further oxidative decarboxylation, alkaline oxidation of lignosulphonates obtained from wood pulp mills or enzymatic bioconversion of ferulic acid. Vanillin also shows biological activity towards a wide branch of organisms including fish, aquatic invertebrates, earthworms or yeast. In this work the effect of different concentration of vanillin on the growth of green microalga *Chlorella* was evaluated.

## Materials and Methods

❖ **Chlorella growth:** *Chlorella* sp. was obtained from Culture Collection of Baltic Algae (CCBA). An inoculum from two month *Chlorella* culture was cultivated in a modified Bold Basal Medium (BBM), in 500 ml round flasks with an initial medium volume of 160 ml, in an incubator (Certomat® BS-T) equipped with a light source (fluorescents lamps, 5 X 18 W) and a shaker (110 rpm). Day/night cycles (16h/8h) were implemented during 11 days of cultivation. Two different concentration of vanillin: 60mg/L and 300 mg/L were present in 160 ml growth media. Vanillin (POCH) was dissolved in media with no addition of organic solvents.

❖ **Biomass density measurement:** Optical density of *Chlorella* biomass in media with different vanillin concentration was determined by taking aliquots of the culture and measuring absorbance (T80+ UV/VIS Spectrometer PG Instruments Ltd) at wavelength 530 nm (OD530).

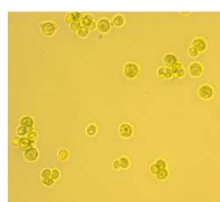
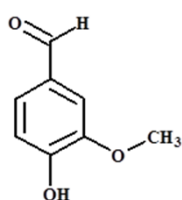
❖ **Chlorophyll extraction and measurement:** Samples of culture aliquots were taken and added to laboratory tubes and centrifugated to remove media. After removing media, pure methanol was added to tubes. When the extraction was complete, aliquots were centrifuged to separate cells from solvent because floating cells cause interferences during chlorophyll measurement. Chlorophyll a and b in obtained extracts were spectrophotometrically measured according to equations:

$$\text{Chla} = (16.72 \cdot A_{665} - 9.16 \cdot A_{652}) \text{ [}\mu\text{g/ml]}$$

$$\text{Chlb} = (34.09 \cdot A_{652} - 15.28 \cdot A_{665}) \text{ [}\mu\text{g/ml]}$$

❖ **Vanillin concentration measurement:** Concentration of vanillin in culture media during *Chlorella* cultivation was evaluated spectrophotometrically by Total Phenol Analysis Method with Folin – Ciocalteu reagent at a wavelength of 765 nm.

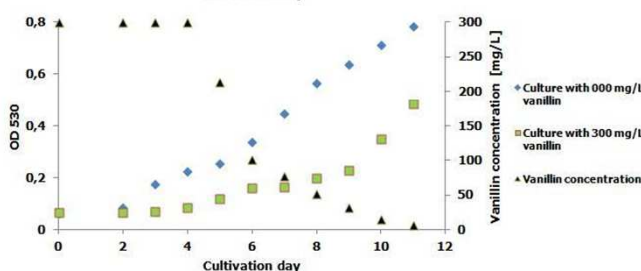
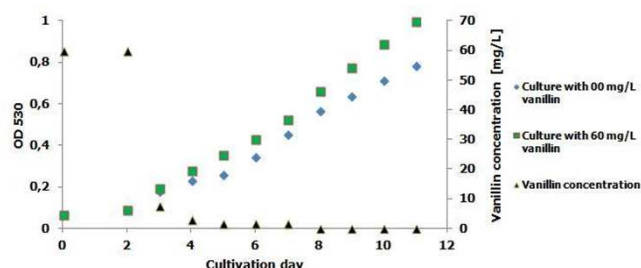
## Results



*Chlorella* sp. from Culture Collection of Baltic Algae (CCBA)

Vanillin concentration [mg/L]	Cultivation days			
	0	4	7	11
0	0.56	3.79	7.00	13.91
60	0.56	4.00	9.52	18.85
300	0.56	≤ 0.56	≤ 0.56	6.52

Chlorophyll content [μg/ml] in *Chlorella* cultures with different amount of vanillin in medium



Biomass growth in *Chlorella* cultures with different amount of vanillin in medium

## Conclusions

- ❖ Vanillin at concentration of 60 mg/L showed to possess stimulating effect on *Chlorella* growth during cultivation, with fast removal of vanillin from cultivation medium.
- ❖ Stimulation of *Chlorella* growth was evaluated due to measurement of biomass and chlorophyll content, which were higher than in control culture.
- ❖ Vanillin at concentration of 300 mg/L showed to exert initial inhibitory effect on *Chlorella* growth with further growth recovery and gradual removal of vanillin from medium.
- ❖ Recovery of *Chlorella* growth was evaluated due to measurement of biomass and chlorophyll content, which were higher than at inhibitory stage of cultivation.
- ❖ Stimulation or recovery of *Chlorella* growth was strictly associated with the decrease of vanillin concentration in medium.