Analysis, on model and real substrates using the available bioreactors from the Faculty of Science and Food Engineering, of the most efficient microorganisms' species able to reduce of the organic compounds released from food industry processes.

Objective IV

Extended abstract

<u>Activity IV.1.</u>

The identification of the most efficient microorganisms able to remove the organic compounds from the food industry wastewaters (dairy and brewery).

Knowing the water quality has a major importance because the industry development leads to the fast increase of the water requirement and of the output wastewater volume from the technological processes.

The indigene and zymogene microorganisms are parts of the wastewater biotic component which constitute a diverse micro-biota, adapted to the physical-chemical specifics, and having an essential role in bioremediation.

The wastewaters from food industry are characterized through a high load of organic compounds and through a large number of microorganisms. The microorganisms from the wastewater are able to degrade the substances from their own environment.

The aim of the study is to evaluate the micro-biota, upon microbiologic criteria, of the wastewaters from the food industry (dairy and brewery) to obtain finally adapted cultures for the biological treatment. The adapted cultures are tested on model and real systems.

The wastewaters from dairies and breweries have a high biodegradable organic load that represents a suitable environment for microorganisms' development. The wastewater samples from Galacta – Galati dairy and from Martens – Galati brewery were used to obtain pure cultures which were microscopic and macroscopic examined to identify the present microorganism categories.

In the dairy wastewater prevail the bacteria and in the brewery wastewater the cultures diversity of bacteria, yeasts and moulds constitute a complex community.

After the microscopic examination, along with cultural particularities, were identified Gram positive and negative bacteria which belong to the *Bacillus* and *Pseudomonas* genus, yeasts from the *Saccharomyces, Torulopsis* and *Kluyveromyces* genus and moulds from the *Aspergillus* and *Geotrichum* genus.

The bioconversion potential of the wastewater micro-biota was determined by cultivating the pure cultures on diverse carbon and nitrogen sources suchlike the ones from the biotope from which the microorganisms were isolated.

There were inoculated cells on specific media from isolated pure cultures which belong to the tree microorganisms' categories. The capacity of metabolizing the starch, maltose and lactose of the bacteria was tested through cultivation on BC media with 1% starch, maltose or lactose.

All the bacteria strains from the brewery wastewater are able to metabolize the maltose. This shows that the micro-biota is adapted to the living media composition, maltose being a carbohydrate which result from the starch hydrolysis found in the malt must and than in beer. The strains IBS1Bc5 and IBS1Bc6 have the best ability to metabolize the maltose.

The capacity to metabolize lactose of the isolated strains was also tested through cultivation on the BC with 1% lactose addition, as only carbon source. After 48 hours of cultivation at 37 ^{O}C a good colonial growth was observed, most strain forming colonies with diameter bigger than 0.5 cm. The IIBS2Bc1 strain developed a colony with 1.2 cm diameter which denotes a good lactose metabolism.

The bacteria capacity to degrade the starch was relieve through „in point" inoculation over the BC media surface with 1% starch (as unique carbon source). After 48 hour of cultivation at 37 ^{O}C and flooding the media surface with 0.1N Lugol solution the starch hydrolysis zone was identified around the active colonies. There were measured the colonies diameters (Dc) and the hydrolysis zones diameters (Dh). The substrate consumption was calculated through the ratio of the two measured diameters (Dh/Dc) named also hydrolysis index (Ih).

All the bacteria strains isolated from the brewery wastewater have the ability to degrade the starch, the best potential being achieved by the IBS1Bc2 strain.

The bacteria were also cultivated on BC media, with 1% casein as unique nitrogen source, for 48 hour at 37 ^{O}C. The growth was point like without a visible hydrolysis zone around the colonies which show a low casein metabolism.

The bioconversion potential over the simple hydrocarbons of the yeasts isolated from the wastewater.

The isolated yeasts strains were tested through the “in point” inoculation technique on DJ media supplemented with 1% lactose or 1% maltose as unique carbon source. After four days of cultivation at 25 ^{O}C the colonial growth was evaluated through the colonies diameters size. The yeasts strains isolated from dairy and brewery wastewaters have a low colonial growth on maltose media, the colonies diameters being smaller than 0.5 cm.

The dairy wastewaters contain lactose as a pollutant compound which is converted, through the micro-biota activity, in lactic acid, butyric acid, propanoic acid and gases.

All the isolated strains are able to metabolize the lactose, but the best potential is showed by the IILBDj3 strain because the colony diameter was 0.8 cm after 4 days of cultivation on 1% lactose media.

The filamentous moulds inferences in the food industry wastewaters organic compounds bioconversion

The moulds pure cultures, isolated from the wastewater micro-biota, presented a low bioconversion potential of the model compounds (starch, casein, simple carbohydrates and lactic acid). The bioconversion potential of these strains was compared with the *Geotrichum candidum* MIUG 1.15 potential, a reliable strain from the Microbiology Laboratory collection.

To identify mould active strains, for constituting a model inoculum useful in the further experiments, the bioconversion potential of the *Geotrichum candidum* from the MIUG collection was studied. There were used five reactivated strains from the Faculty of Food Science and Engineering collection, “Dunarea de Jos” University Galati, with the following codes: MIUG 1.8, MIUG 1.12, MIUG 1.14, MIUG 1.15 and MIUG 1.16. The strains were “in point” inoculated on Czapek media with 1% starch and 1% and 1% lactic acid and then kept for four days at 25 ^{O}C. The colonies diameters, colonies grown on 1% starch media, exceeded 1.0 cm meaning a good growth. The strains MIUG 1.8 and MIUG 1.15 showed the best starch bioconversion potential, those diameters exceeding 2 cm.

The strains MIUG 1.8, MIUG 1.12, MIUG 1.14, MIUG 1.15 and MIUG 1.16 ware “in point” inoculated on agar media supplemented with 1% lactic acid (as unique carbon source) and kept at 25 ^{O}C for four days to monitor the colonial growth.

The experiment was made twice and the diameters average and standard deviation were determined. The MIUG 1.15 strain developed a 1.2 cm diameter, the MIUG 1.14 strain did not grown on lactic acid media and the MIUG 1.12 and MIUG 1.18 had a low colonial growth, those diameters did not exceed 0.5 cm.

Activity IV.2.

Testing of the isolated microorganisms on model substrates prepared in the Wastewater Biological Treatment Laboratory from the "Dunarea de Jos" University Galati.

The aerobic treatment experiments which were leaded on model media pursued the simulation of the wastewaters composition derived from dairy and brewery.

For obtaining the model substrate able to simulate the composition of the dairy wastewater whey was used as substrate. Our source of whey was Galacta dairy from Galati. The physical and chemical properties of the whey used in experiments were the following:

- fats 0%
- density 1.02729 kg/m^3
- lactose 3.81%
- non fat dry substance 7.35%
- proteins 2.92%
- water 18.65%
- cryoscopic point 0.423°C
- mineral salts 0.61%

For the simulation of a model substrate of breweries wastewater a culture media was formed from malt must with hop (150 mL) and clear water, all adjusted with NH_4Cl. The Martens brewery from Galati provided us with malt must with hop representing the organic substrate similar with the wastewater pollutants. The malt must with hop have a high concentration of organic compounds varying between 90.000 ÷ 100.000 mg/l O_2. Most of the organic compounds derived from malt are carbohydrates and, in a small amount, proteins and that is why the concentration of organic nitrogen is very low (CBO_5: N ratio being of 100:1.3). For obtaining an ideal ratio CBO_5: N of 100:5 it was necessary the supplementation with 0.84g NH_4Cl / liter of diluted media. Another macro element of great importance in microorganisms' development is the phosphorus found in large quantities in the malt must with hop. The phosphorus concentration varies between 245 ÷ 260 mg/L.

For pursuing the microorganisms' evolution and their capacity of consuming organic compounds two of the most revealing experiments were analyzed. Both experiments were developed on the 1 liter Aplikon bioreactor and they pursued to provide controlled ideal conditions of treatment. The air quantity blown was set for both experiments at 2 L/min this representing less than the necessary for the microorganisms' physiologic activity, leading to their competition for oxygen therefore for substrate. The pH was also controlled through the addition of acid or base on optimal values for the development of inoculums (pH=5.5 in the first experiment and pH=6 for the second experiment). The working temperature was maintained on a constant value of 25ºC and on the air line a filter was introduced having the purpose of locking the external microorganisms to participate on the process.

The 1st experiment unfolded on a period of 12 days in batch type system, period in which the principal process parameters were registered. The chemical oxygen demand, very high at first, reached in the sixth day the lowest value. The removal efficiency of the organic compounds after 6 days (144h) of aerobically treatment reaches the maximum value of approximately 95%. After reaching this minimal value, the COD grows due to the autolysis of a part from the formed micro-biota (sludge). This point of inflexion is known as *the stabilization point* and represents the maximum limit of organic compounds reduction. This point is characteristic for each system in part.

In practice the stabilization process is named **extended aeration treatment** and is applied afterwards to the proper treatment for reducing the quantity of volatile organic compounds (VOC). The effect of the stabilization is represented through the substantial reduction of the unpleasant smells, the destruction of pathogen bacteria and the obtaining of sludge valuable for agriculture. During the stabilization period the COD may sensitive vary around the last value.

The media turbidity gives precious information about the biomass accumulation in bio-reactor. Through cells multiplication the concentration of biomass grows and therefore the turbidity is changing – is growing. In parallel a reduction of the nitrogen compounds concentration is registered (N_{tot}), because through the substrate biodegradation results assimilable compounds which are used by the cells for the biomass biosynthesis.

In the figure 3.15 is showed the inverse ration between turbidity and COD. As the turbidity increase the number of microorganism grow consuming the organic substances and decreasing the COD. The turbidity decreaseing is due to the autolysis of some microorganisms which become food for the living ones.

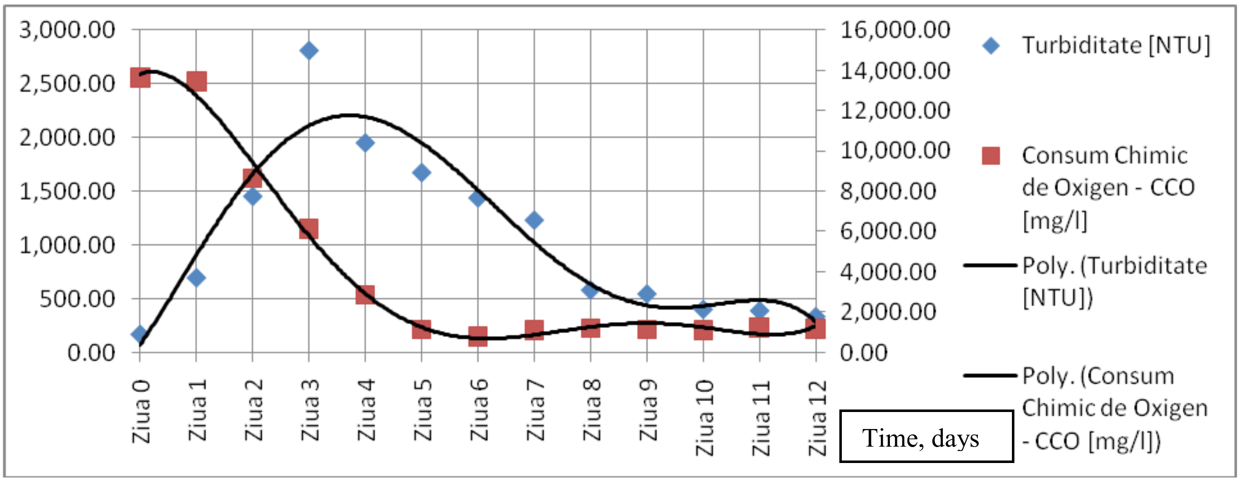


Fig. 3.15. Turbidity and COD inverse ratio during the biological treatment of a whey test model with specialized inoculum. (process realized in the 1L bioreactor)

Also the turbidity is in inverse ratio with the dissolved oxygen as it can be seen in the figure 3.16. The blown air volume is constant thereby the increase of microorganisms concentration lead to a deeper oxygen consumption and therefore the dissolved oxygen decrease.

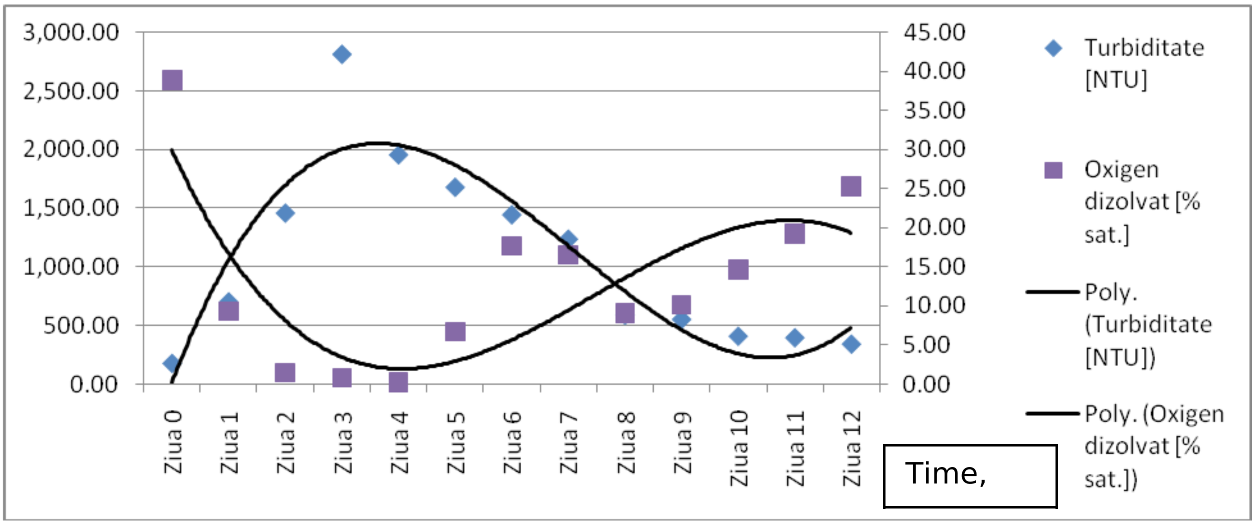


Fig. 3.16. Turbidity and DO inverse ratio during the biological treatment of a whey test model with specialized inculum. (process realized in the 1L bioreactor)

If the turbidity is in inverse ratio with COD and DO it means that the last two are in direct ratio as we can see in the figure 3.17.

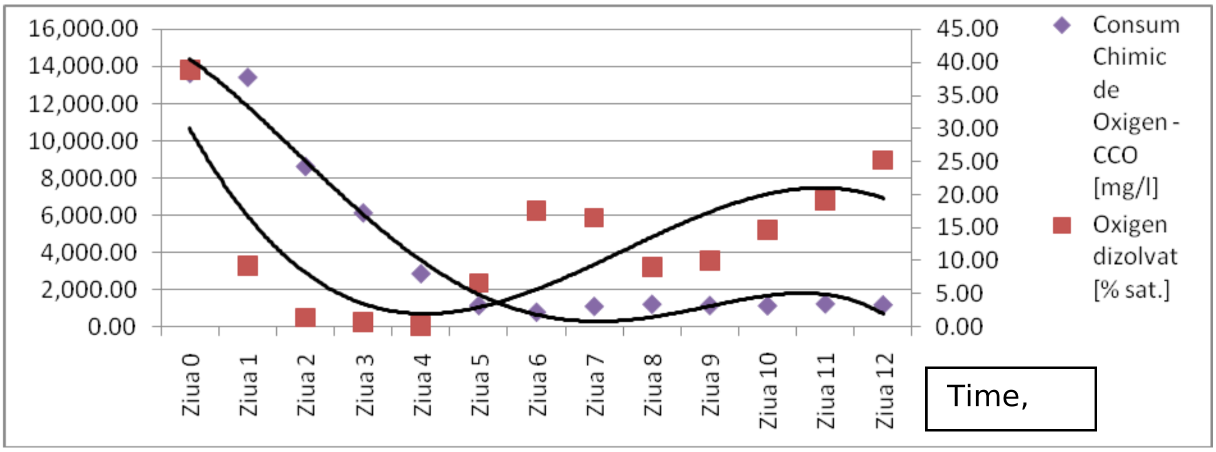


Fig. 3.17. COD and DO direct ratio during the biological treatment of a whey test model with specialized inoculum. (process realized in the 1L bioreactor)

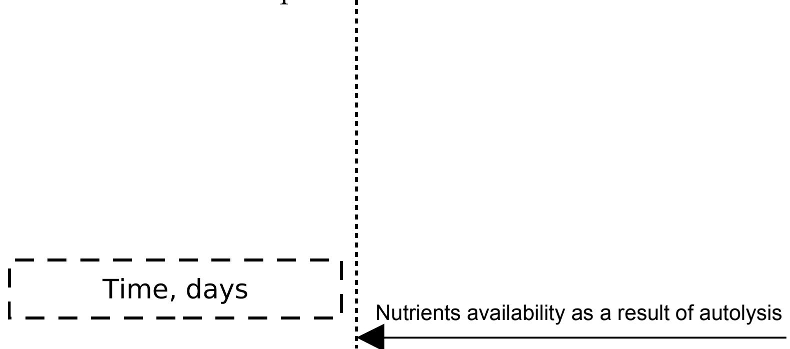
Because the COD analysis is off-line and take couple of hours, the monitoring of the turbidity and DO represent a rapid system to control the COD in aerobic treatment installations.

The second experiment was made on malt must with hop diluted 1:5 with clear water, with an initial COD load of 19.700 mg O_2/L and a specialized inoculum. This is also a batch experiment roll on nine days. In this case the COD removal is very high; the removal efficiency after 9 days was 90%.

The N_{tot} and $P\text{-}PO_4^{3-}$ evolution are unusual, the nitrogen is completely reduced in the second day and the phosphorus is totally removed until the 4th day of treatment. After the complete removal of the two macro-elements the organic carbon concentration is still 50% thereby the remaining compounds are formed of C, O and H (starch, dextrin and simple carbohydrates). The availability of the nitrogen (5th day) and phosphorus (7th day) in the culture media means that some microorganisms were autolyzed.

The complete assimilation of the nitrogen and phosphorus is important for the treated water but is not convenient for the process itself because their absence restrains, or at least delay, the microorganisms' development.

The correlation between turbidity and COD is affected by this point of inflexion as it can be seen in the figure 3.23. The direct ratio between COD and turbidity is valid just for the period in which the nutrients are presents in the culture media.



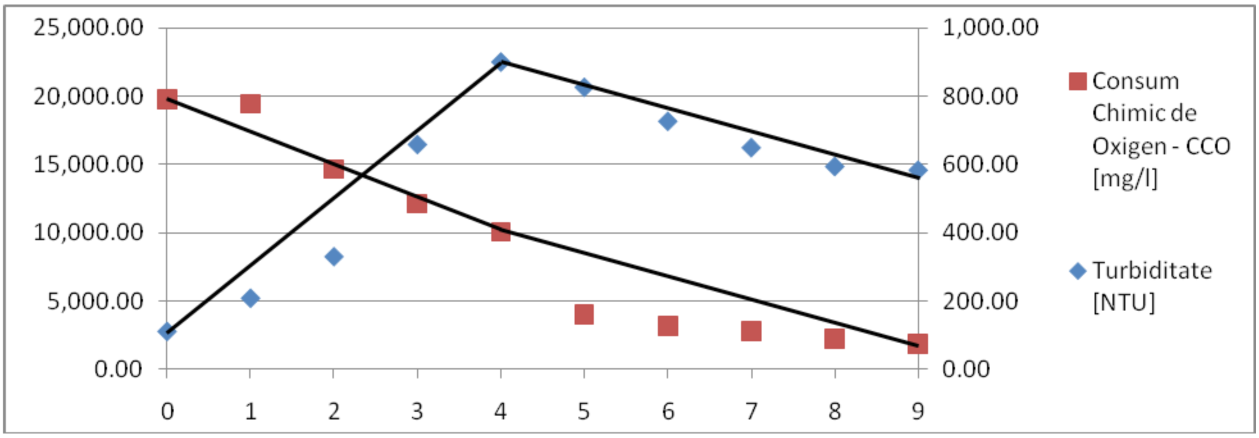


Fig. 3.23. COD – Turbidity correlation during the biological treatment of a whey test model with specialized inoculum. (process realized in the 1L bioreactor)

The DO and turbidity are again in inverse ratio, but this correlation is also affected by the nutrients evolution (figure 3.24).

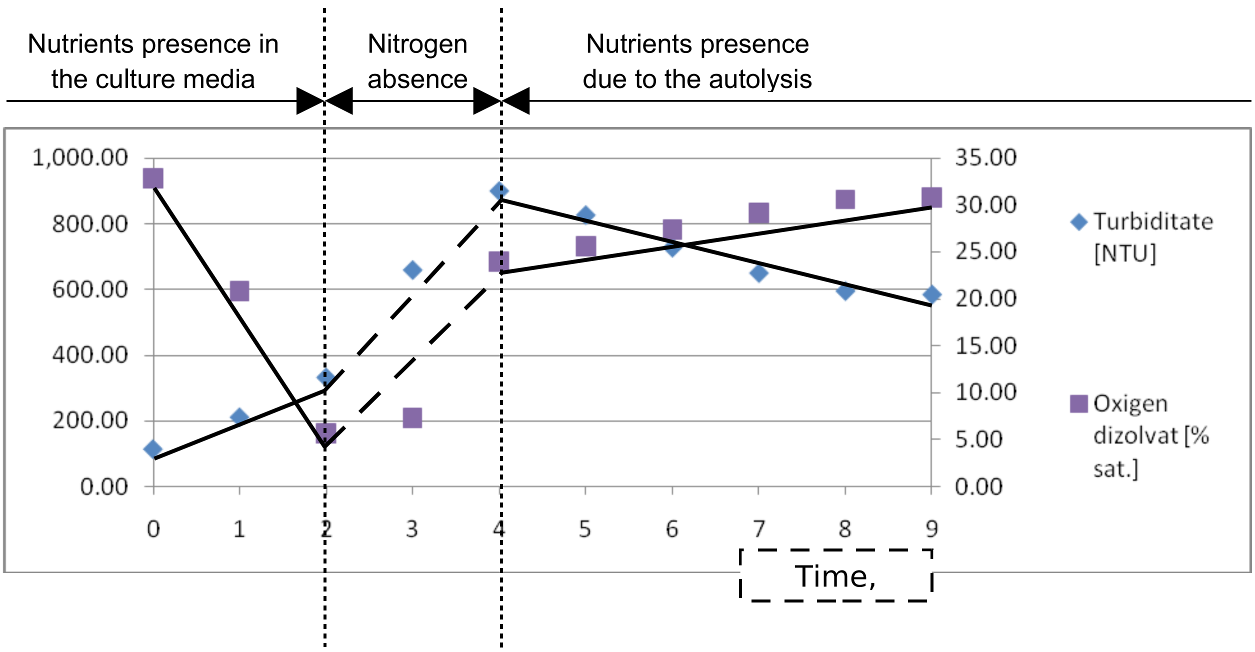


Fig. 3.24. DO – Turbidity correlation during the biological treatment of a whey test model with specialized inoculum. (process realized in the 1L bioreactor)

Like in the previous experiment the COD can be correlated with the DO. This correlation is also affected by the nutrients evolution (figure 3.25)

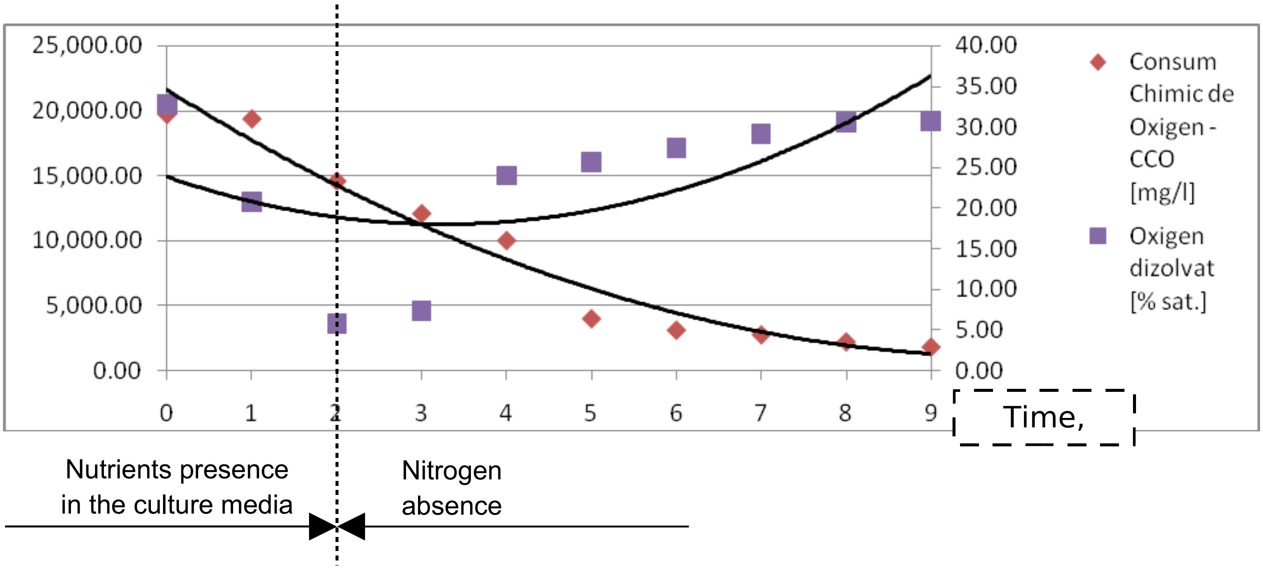


Fig. 3.25. COD – DO correlation during the biological treatment of whey test model with specialized inoculum. (process realized in the 1L bioreactor)

To economically improve the process the air flow can be reduced, but a better solution is to control the nutrients (N and P) which warrant a shorter treatment period.

<u>Activity IV.3.</u>

Testing of the isolated microorganisms on real substrates provided from food industry factories (dairy and brewery).

In an aerobic treatment process of the wastewaters, the organic substances (pollutants) are transformed into biomass, being oxidized by the oxygen from the blown air. The aerobe respiration is a process that generates ATP which is involved in the electrons transportation. The following experiments are batch type that uses just the aeration tank for all the operations. This system is also called Sequence Batch Reactor.

The third experiment was conducted on whey culture media diluted 1:4 with clear water at an initial concentration of 12.000 mg O_2/L. The inoculum was compiled from the following microorganisms: IILGBc2 (bacteria), ILBDj3 (yeast) and MIUG 1.15 (mould, *Geotrichum candidum*). The total inoculated volume was 1.4 L; 4% (v/v) concentration in comparison with the bioreactor working volume.

The values of pH, DO and ORP was measured automatically at every 10 seconds with the electrochemical sensors submerged into the aeration tank.

The bacteria grow at pH which varies between 4.5 ÷ 11 with an optimum of 6.5 ÷ 7.5 while the yeasts and moulds develop at pH between 1.5 ÷ 8.5 with an optimum of 4.5 ÷ 6.5. In the third experiment the pH varied between 5.76 ÷ 7.68 meanings that the dominant microorganisms were the bacteria. Also the high values of the turbidity show the bacteria domination.

The turbidity is (like in the previous experiments) in inverse ratio with the COD as it can be seen in the figure 3.36. The minimum COD value is reached after 78 hours and beyond this point the COD grows. This point is called stabilization point and has the signification of a technical treatment limit of the plant. The removal efficiency in the stabilization point is 98.8% away better toward the 1L bioreactor experiment.

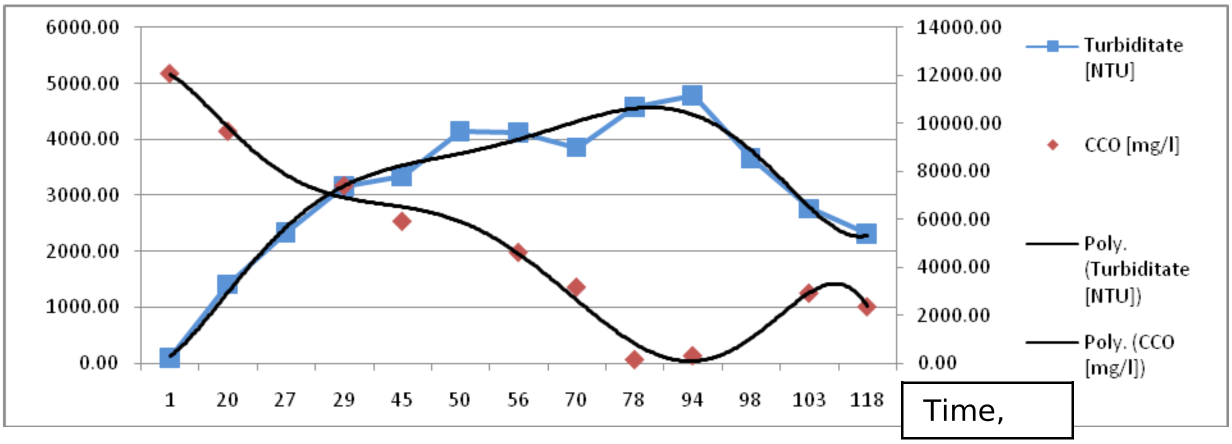


Fig. 3.36. Turbidity and COD inverse ratio during the biological treatment of dairy wastewater on the pilot plant using a specialized inoculum with bactera, yeasts and moulds

Like in the previouses experiments the turbidity is also in inverce ratio to the DO, but, because of the low values of the DO, the corelation can not be graphically showed.

Another important parameter is the oxido-reducing potential (ORP). When the DO is closed to zero the ORP can give useful informations about the process because it can take negative values. In practice the corellation between ORP and DO is well known, but in this case it is affected by the stabilization point as it is showed in the figures 3.38 and 3.39.

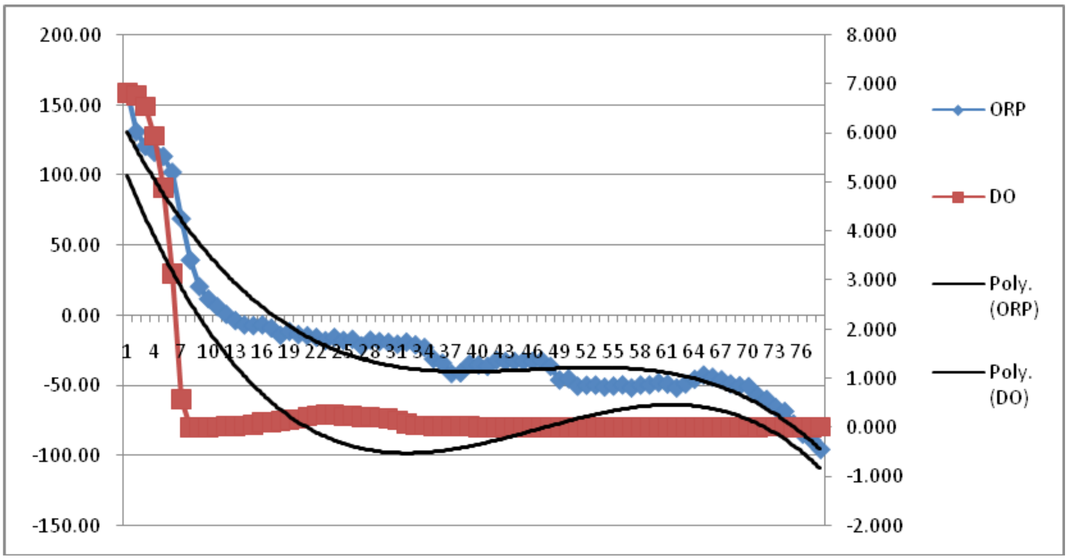


Fig. 3.38. ORP - DO direct ratio <u>before the stabilization point</u> during the biological treatment of dairy wastewater on the pilot plant using a specialized inoculum with bactera, yeasts and moulds

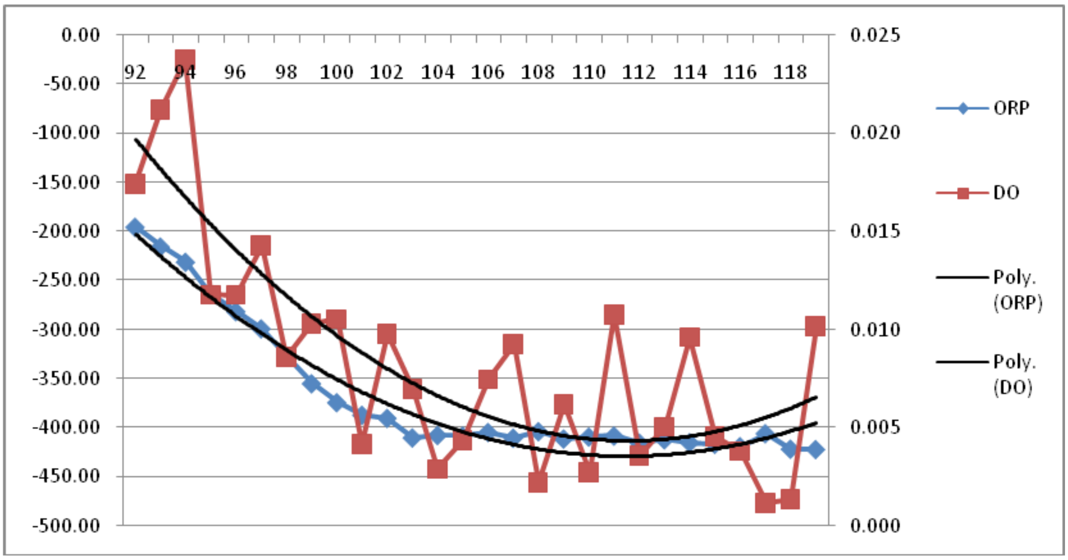


Fig. 3.39. ORP - DO direct ratio <u>after the stabilization point</u> during the biological treatment of dairy wastewater on the pilot plant using a specialized inoculum with bactera, yeasts and moulds

If the correlation between turbidity and DO could not be graphically represented, the correlation between turbidity and ORP can be seen in the figure 3.40. The evolution of the both curves is affected of the stabilization point.

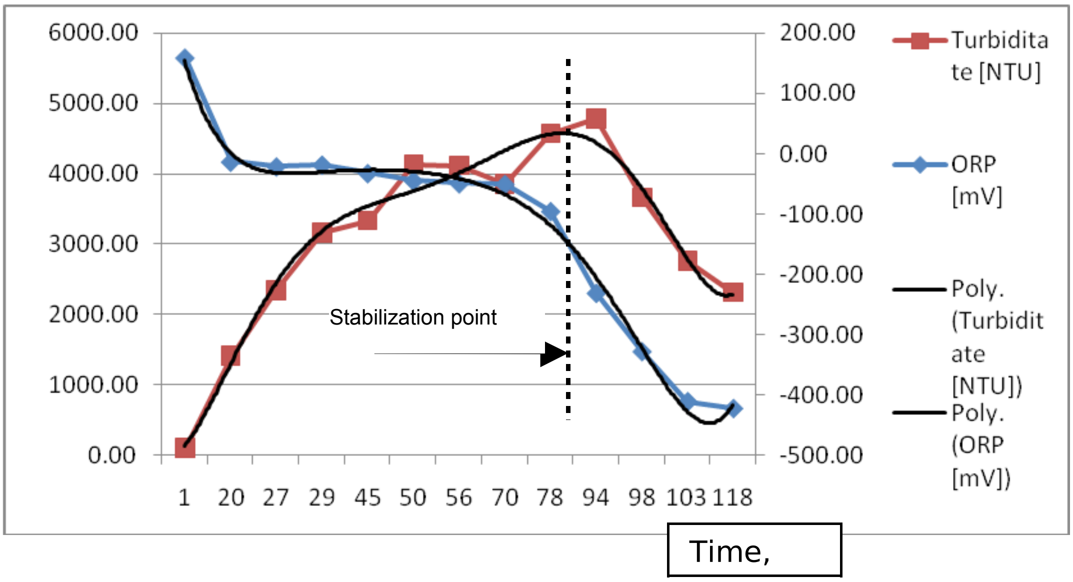


Fig. 3.40. Turbidity - ORP correlation during the biological treatment of dairy wastewater on the pilot plant using a specialized inoculum with bactera, yeasts and moulds

If the turbidity is in inverse ratio with COD, DO and ORP than we can build the direct ratio between COD and ORP (figure 3.41).

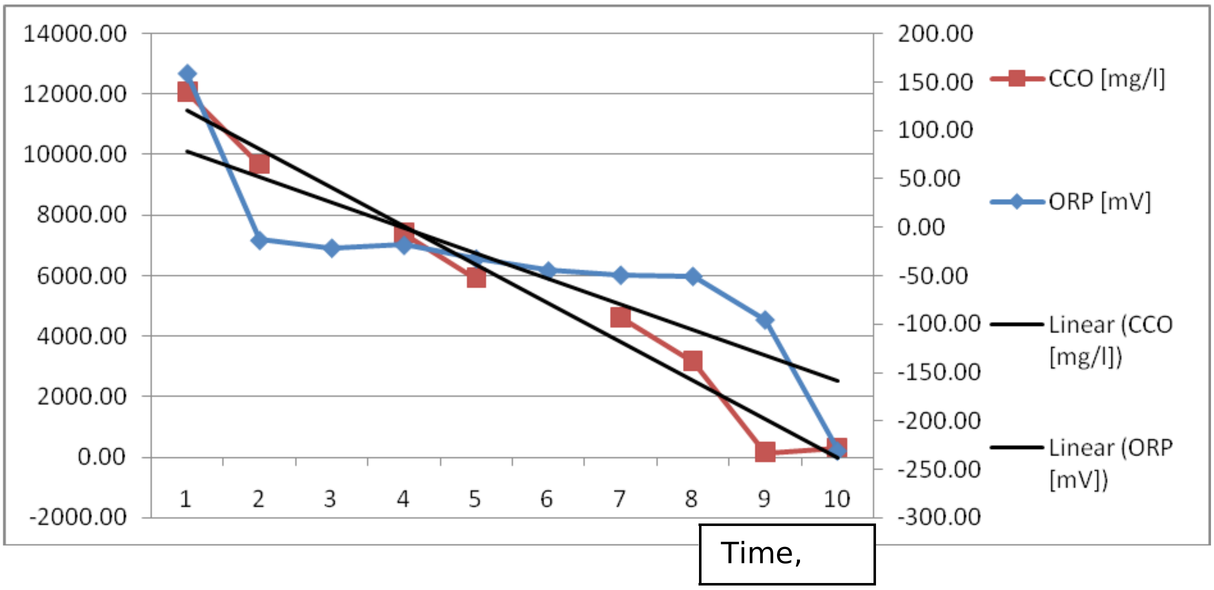


Fig. 3.41. ORP - COD direct ratio during the biological treatment of dairy wastewater on the pilot plant using a specialized inoculum with bactera, yeasts and moulds

The correlation between ORP and COD is important because the COD is an off-line analysis that take a lot of time and the ORP measurement is on-line and involve cheap instruments. The correlation is valide on bach type systems.

During this experiment the COD, N_{tot} and PO_4^{3-} removal efficiencies in the stabilization point are:

- CCO: 98.8%
- N_{tot}: 63.6%
- PO_4^{3-}: 46.1%

The fourth experiment was leaded on malt must with hop; diluted 1:8 with clear water, with an initial COD concentration of 11.600 mg O_2/L. The culture media was supplemented with NH_4Cl because the low nitrogen concentration.

In this experiment the pH was lower comparing to the previous experiments, its value varying around 4.7 units with a very small variation, 0.11 units. Because of this low value we can appreciate that fungi are the dominant microorganisms. Also the turbidity shows that the dominant microorganisms are the fungi because its values are smaller than previous. In the figure 3.52 and table 3.8 the turbidities of the third and fourth experiments are compared; the maximum turbidity in the third experiment is 4787.5 NTU and in the fourth only 2215.5 NTU. These results explain the variability of the aerobic treatment biochemical processes in correlation with the chemical composition of the wastewaters, the pollutants type, the involved microorganisms, the adaptability degree and the competition between the species.

Table. 3.8. Turbidity variation during the 3rd and 4th experiments

	Turbidity variation during the aerobic treatment									
	1	2	3	4	5	6	7	8	9	10
Exp 3 (dairy wastewater)	Time, hour									
	1	20	29	45	56	70	78	94	103	118
	97	1413	3163	3338	4115	3854	4575	4788	2765	2312
Exp 4 (brewery wastewater)	Time, hour									
	1	21	30	46	56	71	79	95	102	118
	97	430	735	1395	1627	1935	2038	2223	2248	2215

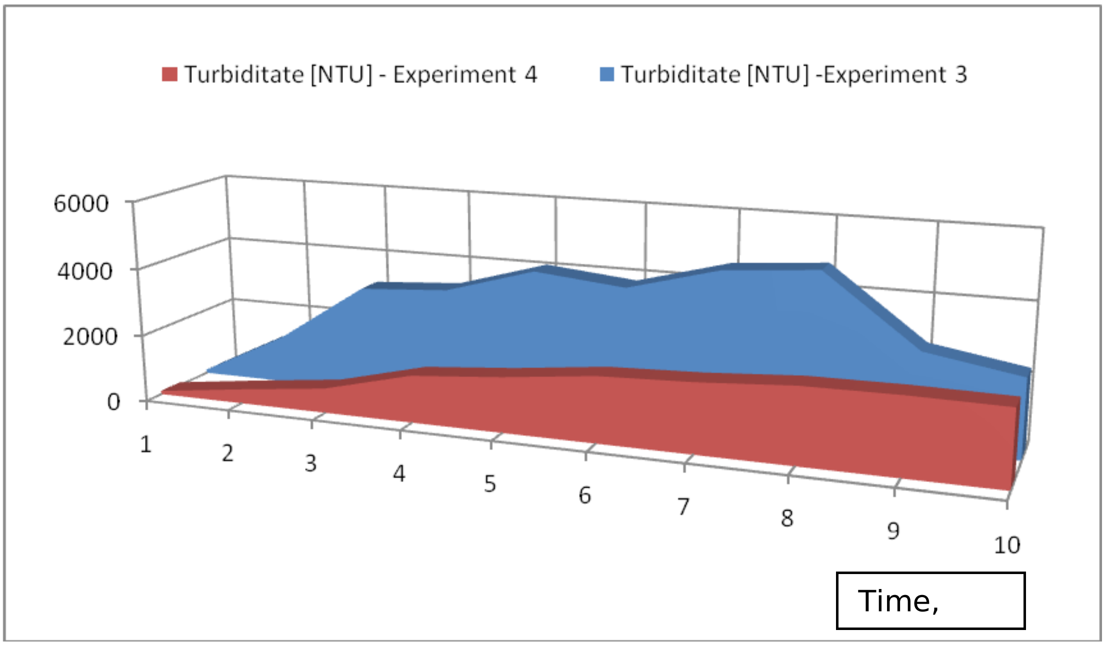


Fig. 3.52. Turbidity variation during the 3rd and 4th experiments

The COD removal efficiency reached 73% but the effluent load was still high (3124 mg/L). Like in the previous cases the turbidity is in inverse ratio to the COD as it can be seen in the figure 3.53. The correlation between COD and turbidity is very high, 98% (calculated with the "CORREL" function from Microsoft Excel)

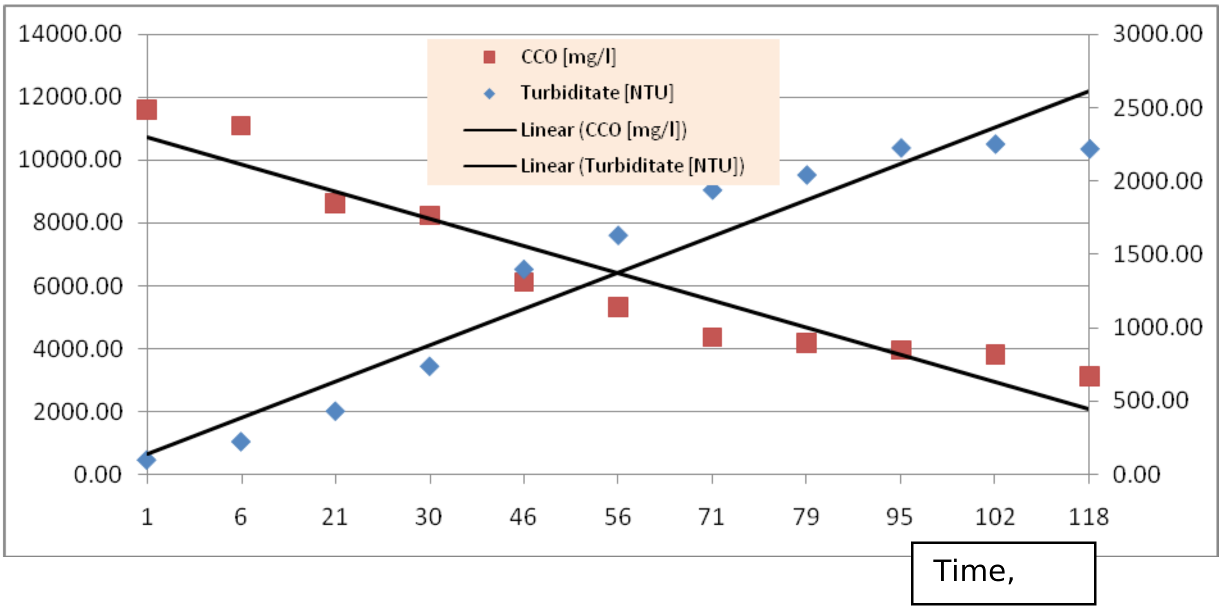


Fig. 3.53. COD – Turbidity inverse ratio during aerobic treatment of a brewery wastewater

The ORP registered an opposite tendency during this experiment in comparison with the priors, but the direct ratio to the DO remained valid (figure 3.54.). The DO was set at different values: 2, 3, 4 and 5 mg/L; was observed that the blown air volume was smaller in comparison with the dairy wastewater.

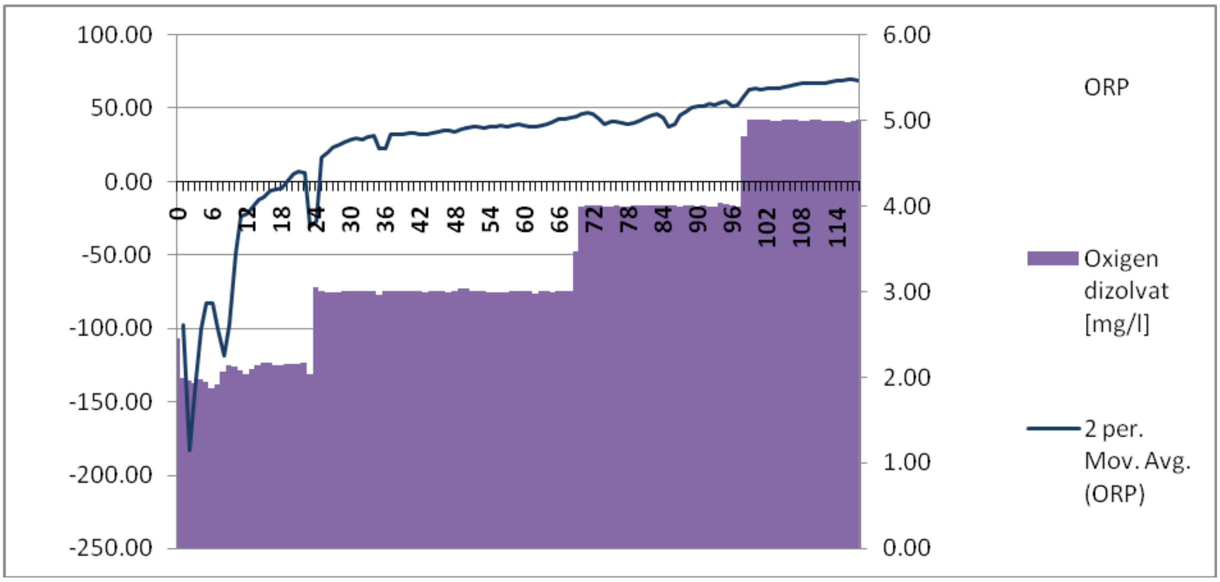


Fig. 3.54. ORP and DO evolution during aerobic treatment of brewery wastewaters

In the present experiment the correlation between ORP and COD is in inverse ratio but it cannot be considered valid. This tendency can be natural for the specialized inoculumn cultivated in this type of wastewater and also can be a result of the dissolved oxygen decrease.

In real conditions the removal efficiencies for COD, N_{tot} and PO_4^{3-} are:

- CCO: 78 %
- N_{tot}: 73 %
- PO_4^{3-}: 99 %

<u>Activity IV.4.</u>

Measurements in dynamic regime for qualitative indicators evaluation during aerobic wastewater treatment.

The aerobic wastewater treatment consists in the transformation of the organic matter in CO_2, H_2O, NH_4 and biomass. A conventional dynamic process consists in an aerated tank and a clarifier. The biomass (sludge) settled at the bottom of the clarifier is recycled back to the aerated tank to keep a growing or constant microorganisms concentration in the tank.

In the following experiments the wastewaters were inoculated with sludge obtained from the yeast factory Rompak – Pascani. The sludge is formed from mixed populations of bacteria, yeasts, moulds and protozoa which are grouped in flocks.

The 5th experiment last for 91 hours and was leaded on dairy wastewater. The purpose of this experiment was to impose some dynamic modifications over some vital parameters as air flow and feeding flow and to identify a treatment model. These dynamic modifications have influence over almost all parameters. The following modifications were made:

1. 9th hour the air flow was increased from 5 L/min to 10 L/min
2. 21st hour the air flow was decreased from 10 L/min to 8 L/min
3. 50th hour the feeding flow was increased from 2.2 L/h to 4.5 L/h
4. 67th hour the air flow was increased back to 10 L/min

All these four modifications can be seen on the pH evolution curve (figure 3.65).

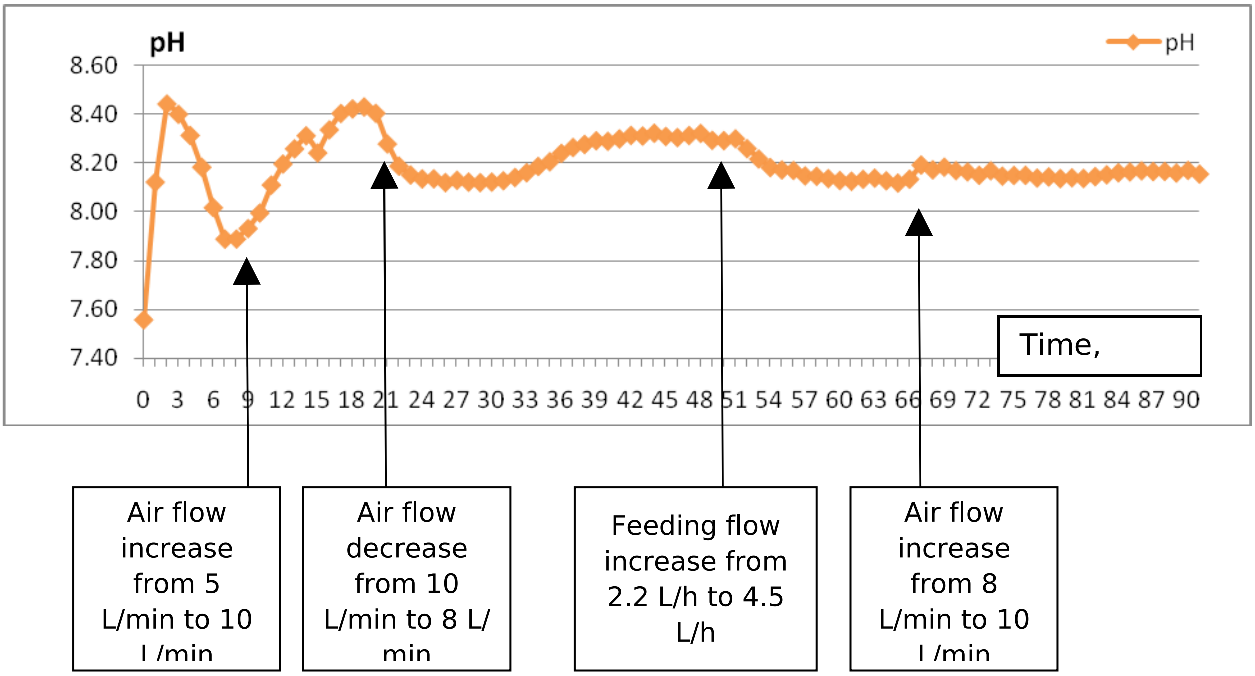


Fig. 3.65. pH response to the dynamic modification during aerobic treatment of dairy wastewaters

All the previous correlations between the measured parameters (on dairy wastewater) remain valid but there are influenced by the imposed modifications.

As in the previous experiments the turbidity can be correlated with the pH, COD, DO and ORP. The correlation between removed COD and ORP remains also valid but it can be applied just in strict working condition because the response time of ORP to substrate modification is very slow. In the figure 3.72 the direct ratio between removed COD and ORP is graphically showed but just for the first 76 ore because the last modification impose a different evolution between the two of them.

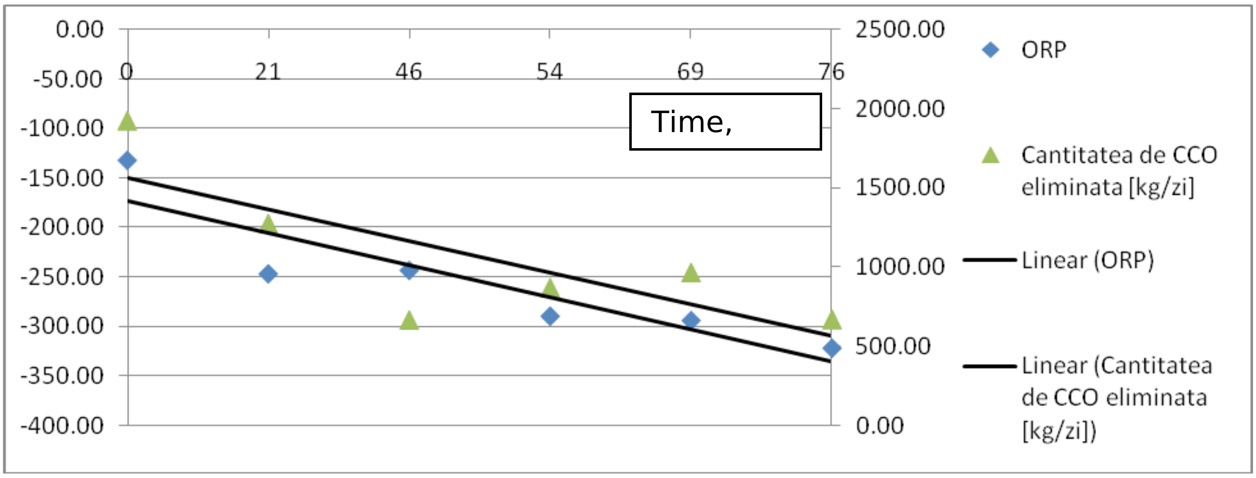


Fig. 3.72. The direct ratio between COD and ORP for the first 76 hours at the aerobic treatment of the dairy wastewater with the recycling of the sludge

The 11th and 12th experiments was made on malt must with hop and the both last for 49 hours. The culture media dilution was 1:60 with clear water. The difference between the two was the nitrogen addition, thus:

- Experiment 11th: worked without NH_4Cl addition during the first 24 hours and with NH_4Cl addition during the last hours;
- Experiment 12th: worked with NH_4Cl addition during the first 24 hours and without NH_4Cl addition during the last hours.

As it can be seen in the 3.76 figure, in the 12th experiment, the turbidity decreases dramatically after the ceasing of the nitrogen addition. The nitrogen is an important macroelement for the metabolic activity of the microorganisms, being constituent of the proteins, enzymes, nucleic acids etc.

In opposition, in the 11th experiment the microorganisms has adapted to the minimal initial conditions with low nitrogen concentration and its addition on the last hours imposed an exponential increasing of the turbidity.

In comparison with the 4th experiment in which the ORP took positive values, in the 11th and 12th experiments the ORP had negative values and this is a result of the microorganisms' diversity from the sludge and low COD concentration.

During the 12th experiment the DO value was set to 2 mg/L using a regulator. The result of this DO regulator was a 3 time less air consumption in comparison with the 11th experiment in which the air inlet had a fixed value.

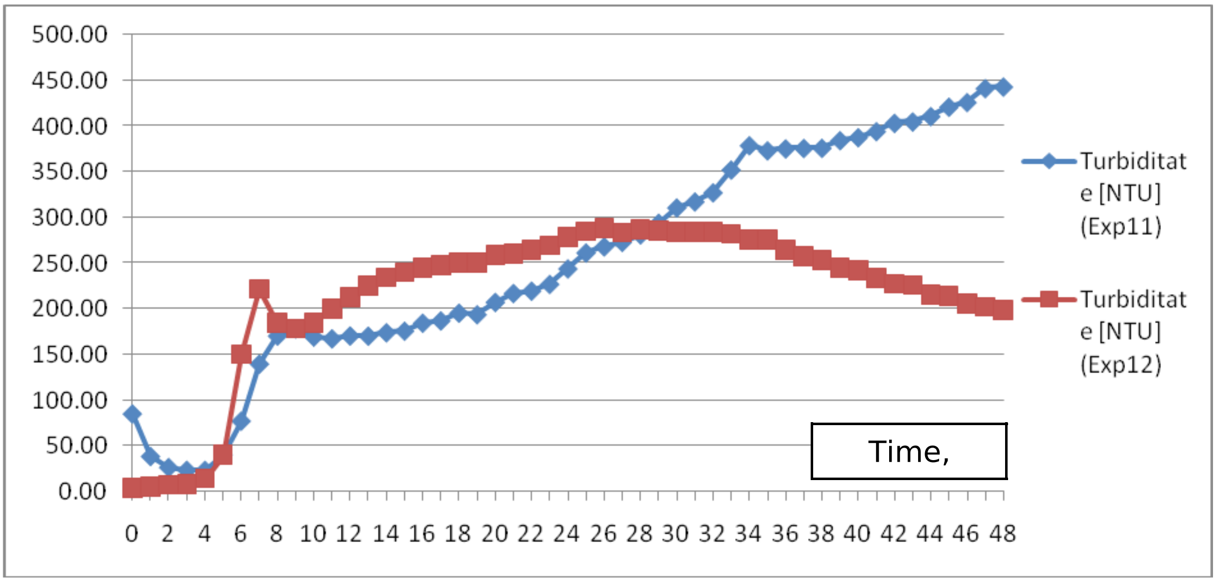


Fig. 3.76. The turbidity evolution in the 11th and 12th experiments during aerobic treatment of the brewery wastewater.

With the 15th experiment there was introduced a new control parameter, food to microorganisms ratio. The objective of this experiment was to keep constant the F/M ratio on different levels. This experiment last for 54 hours on dairy wastewater. The F/M ratio is defined as the ratio between the quantity of substrate and the quantity of microorganism and it is calculated with the following formula:

$$F/M = \frac{Q \cdot 24 \cdot BOD_5}{TSS \cdot V} \ [kg\ CBO_5/kgTSS \cdot zi]$$

Were:

Q = wastewater feeding flow [L/h]

BOD_5 = wastewater biochemical oxygen demand concentration [mg/L]

TSS = total suspended solids in the aeration tank [mg/L]

V = aeration tank working volume [L]

The BOD_5 analysis on the dairy wastewater showed that the load was 1000 mg/L. To correlate the optical turbidity measurement with the conventional gravimetric TSS there were made parallel off-line measurement. To obtain a real TSS value the continuously measured turbidity must be multiplied 4 times.

There were imposed 3 different fixed values for F/M ratio, as follows:

- F/M = 2.4 kg BOD_5/kg TSS * day, in the first 34 hours
- F/M = 1.2 kg BOD_5/kg TSS * day, in the next 11 hours
- F/M = 0.7 kg BOD_5/kg TSS * day, in the last 9 hours

As the F/M ratio, BOD_5 and working volume are constant it can be obtained the following relation:

$$Q = f(Turbidity)$$

Applying this control law the wastewater flow will increase along with the turbidity. As a result of the F/M ratio control the pH and ORP profiles are more linear in comparison with the previous experiments. In this way it can be accentuate that the ORP depend of COD, turbidity and pH in the same time.

The quantity of COD removed depends on the number of microorganisms form the aeration tank and also of the F/M ratio as it can be seen in the figure 3.88.

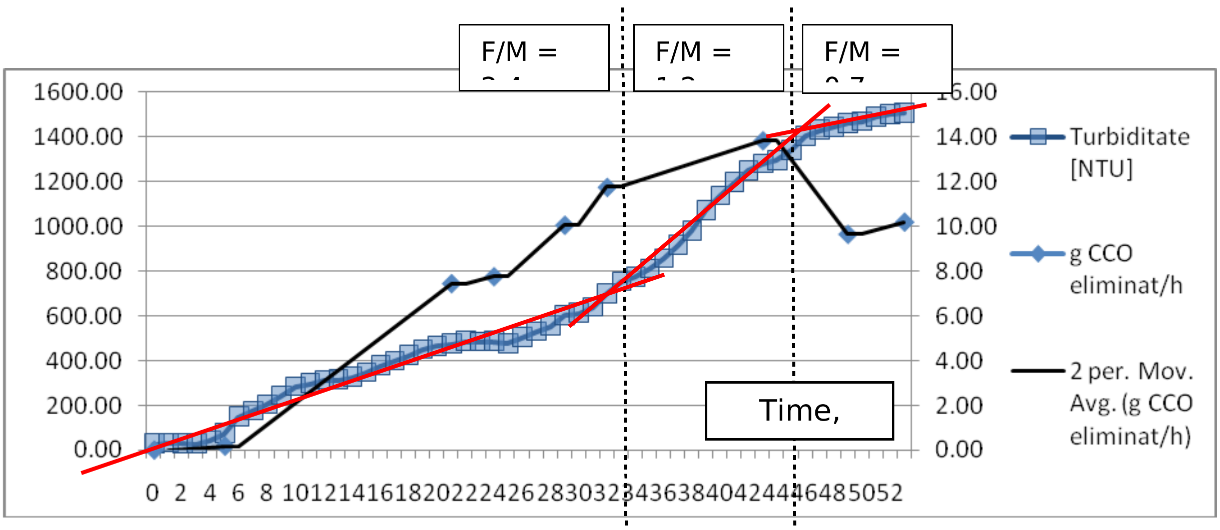


Fig. 3.88. Correlation between turbidity and removed COD, at different F/M ratio levels, on the aerobic treatment of the dairy wastewater.

In the first 34 hours the turbidity reached 650 NTU increasing linearly and along with this the quantity of COD removed. The COD removal at the end of the first 34 hour is just 50%. In the next 11 hours the F/M ratio was decreased from 2.4 to 1.2 kg BOD_5/kg TSS * day resulting in an accelerate turbidity increasing and reaching a 2 times bigger value (~1300 NTU) in a 3 time smaller time interval. Therefore a high organic load in the wastewater have an inhibitory effect over the biomass development. At the end of this interval was reached the biggest quantity of removed COD, the efficiency increasing with 14%. To obtain better efficiency the F/M ratio was decreased at 0.7 kg BOD_5/kg TSS * day. The efficiency increased with 6% but the quantity of COD removed decreased. During this interval the turbidity increased only with 150 NTU, this phenomenon being explained through the rapid consumption of a certain substrate, the competition between the species and the high microorganisms' density in the pool.

The outlet COD level is very important for the treated water evacuation in rivers or municipal sewage and for this a compromise in favor of COD removal efficiency and in detriment of the quantity of COD removed has to be made. The quantity of COD removed is characteristic to each plant and a balance between COD load and COD quantity has to be found.