Belgrade, 02.12.2024

Slobodanka Miklja,

Project Leader, Supervisor/ Manager

Dear Slobodanka, PR- CTO

In accordance with our previous agreement, we are enclosing the results of the testing conducted on the submitted sample named Pikua Rense Sanitizer, intended as an agent for improving washing and cleaning, as well as for oxidation/wastewater treatment.

Introduction to Wastewater Parameters: COD, BOD, and TOC

Wastewater contains various organic and inorganic pollutants, and its characterization is critical for environmental management. Three key parameters are commonly used to assess organic pollution: Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), and Total Organic Carbon (TOC).

1. **Chemical Oxygen Demand (COD):**
   COD measures the amount of oxygen required to chemically oxidize organic and inorganic matter in water. It originates from industrial effluents, domestic wastewater, and agricultural runoff. High COD levels indicate significant pollution.
2. **Biological Oxygen Demand (BOD):**
   BOD reflects the amount of oxygen consumed by microorganisms during the decomposition of organic matter over a specific period (usually five days). It is primarily associated with biodegradable organic pollutants from household waste, food industries, and natural organic detritus.
3. **Total Organic Carbon (TOC):**
   TOC quantifies the total concentration of organic carbon in water, providing a broader measure of organic content. It includes both biodegradable and nonbiodegradable fractions, originating from natural organic matter, industrial discharges, and synthetic chemicals.

Classification Based on Parameters

- **Low pollution:** BOD < 3 mg/L, COD < 20 mg/L, TOC < 2 mg/L (e.g., treated wastewater, pristine water bodies).
- **Moderate pollution:** BOD 3–10 mg/L, COD 20–50 mg/L, TOC 2–10 mg/L (e.g., urban wastewater).
- **High pollution:** BOD > 10 mg/L, COD > 50 mg/L, TOC > 10 mg/L (e.g., untreated industrial and municipal wastewater).

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Reduction and Removal Techniques

- **COD and BOD:** Effective methods include aerobic and anaerobic biological treatment (e.g., activated sludge, biofilm reactors) and advanced oxidation processes (e.g., ozone, Fenton reaction).
- **TOC:** Physical-chemical techniques such as coagulation, adsorption (e.g., activated carbon), and membrane filtration are commonly used. Thermal oxidation methods can also efficiently reduce TOC in industrial effluents. Efficient management of these parameters is crucial for mitigating environmental pollution and ensuring compliance with regulatory standards.

In all experiments we used standard analytical methods

1. **Chemical Oxygen Demand (COD):** ISO 6060 - *Water quality — Determination of the chemical oxygen demand*.

2. **Biological Oxygen Demand (BOD):**
   ISO 5815-1 - *Water quality — Determination of biochemical oxygen demand after n days (BODn) — Part 1: Dilution and seeding method with allylthiourea addition*. ISO 5815-2 - *Water quality — Determination of biochemical oxygen demand after n days (BODn) — Part 2: Method for undiluted samples*.
3. **Total Organic Carbon (TOC):**
   ISO 8245 - *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*.

For experiment we used HIGH pollution water with NO heating and 40C heating, and in both cases 15 minutes of contact/reaction time

	Initial	0.5 g/L	1g/L	2g/L	4 g/L
COD	60	52	45	25	5
BOD	20	16	10	8	2
TOC	15	15	12	10	7
COD 40C	60	50	40	15	3
BOD 40 C	20	14	8	5	1
TOC 40C	15	14	10	5	4

The results clearly demonstrate that oxidation potential, rapid solubility in water, and the influence of temperature on oxidation in wastewater present significant potential for the application of a PR sanitizer in wastewater treatment.

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Laundry Washing Performance Testing with WFK Fabrics

Laundry washing performance testing is an essential procedure to evaluate the cleaning efficiency of detergents and washing processes. A widely accepted method involves the use of standardized Wissenschaftliche Forschungsstelle für Textiltechnik und Pflege e.V. (WFK) test fabrics. These fabrics are specifically designed to simulate real-world soiling and staining conditions, allowing for reproducible and comparative testing.

What Are WFK Test Fabrics?

WFK test fabrics are standardized materials pre-soiled with specific stains that represent common household or industrial contaminants. They are developed to test various aspects of laundry detergents, such as stain removal, color preservation, and fabric care.

The stains include:

- **Protein-based stains:** Blood, milk, and egg residues.
- **Oil-based stains:** Cooking oils or cosmetics.
- **Pigmented stains:** Coffee, wine, or tea.
  The standardized nature of WFK fabrics ensures consistent and comparable results across different testing environments.

Testing Procedure

1. **Preparation:** The test fabrics are cut into specific dimensions and paired with similar unstained control fabrics.
2. **Washing Process:** The fabrics are washed under controlled conditions, typically using standardized washing machines, water hardness levels, and detergent dosages. ISO 6330 (*Textiles — Domestic washing and drying procedures for textile testing*) is often referenced for procedure guidelines.
3. **Evaluation:** After washing, the fabrics are visually and instrumentally assessed for stain removal efficiency. This is commonly done using spectrophotometers to measure changes in reflectance or color coordinates before and after washing.

Significance of WFK Test Fabrics

- **Standardization:** Ensures uniformity in testing and comparability of results.
- **Versatility:** Suitable for testing various detergents, formulations, and washing technologies.
- **Benchmarking:** Provides a reliable way to compare the performance of new products with existing market standards.

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Applications

- **Product Development:** Used by detergent manufacturers to optimize formulations.
- **Quality Control:** Ensures that detergents meet performance criteria.
- **Consumer Testing:** Provides data for claims about stain removal efficiency.

The use of WFK test fabrics is crucial in establishing a scientific basis for evaluating and improving laundry detergents and processes.
In experiments we used standard procedures such as

1. ***ISO 6330*** *Textiles — Domestic washing and drying procedures for textile testing*

2. ***ISO 105-C10***
   Textiles — Tests for color fastness — Part C10: Color fastness to washing with soap or soap and soda

3. ***ISO 3175***
   Textiles — Professional care, dry-cleaning and wet cleaning of fabrics and garments

4. ***IEC 60456***
   Clothes washing machines for household use — Methods for measuring the performance

**EU's current top quality laundry detergent BRAND was determined to be a German product - COMPACT powder product (100 gram/washing cycle) used in testing. This research used 100g of the laundry detergent as the baseline in the washing cycles at various temperatures, then conducted similar testing under the same conditions to examine the results with the addition of 6g of Pikua Rense Sanitizer.*

Washing performances BASE LINE, TOP BRAND 100g/washing cycle

	30C	***40C***	***60C***	***90C***
Tenside effect	26.1	27.6	29.7	35.8
Bleach effect	36.5	36.6	38.8	47.8
Enzyme effect	25.6	26.2	27.1	33.0
Whiteness 10A	83.5	83.5	83.5	83.5
Color care BB	2.40	2.52	3.20	No color
Color Care DR	2.40	2.52	3.30	No color
Color Care SY	2.41	2.44	3.20	No color
Color care MG	2.41	2.54	3.20	No color
Dye uptake 10A	73.3	73.3	73.4	73.0

Washing performances BASE LINE, TOP BRAND 100g + 6g Pikua Rense Sanitizer

	30C	***40C***	***60C***	***90C***
Tenside effect	26.5	28.2	30.3	37.9
Bleach effect	38.2	40.1	42.1	50.1
Enzyme effect	25.7	26.4	27.3	33.4
Whiteness 10A	83.8	83.8	83.8	83.8
Color care BB	2.40	2.52	3.20	No color
Color Care DR	2.40	2.52	3.30	No color
Color Care SY	2.41	2.44	3.20	No color
Color care MG	2.41	2.54	3.20	No color
Dye uptake 10A	73.3	73.3	73.4	73.0

BIOCIDAL ACTION

(*Important information for use in settings such as hospital, hotel, institution, major stadiums, hospitality & manufacturing of food and other similar facilities).

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LOG OF REDUCTION BASE LINE TOP BRAND 100g washing detergent only				
	30C	***40C***	***60C***	***90C***
Gram positive bacteria	2.2	3.0	4.1	6.5
Gram negative bacteria	2.0	2.8	4.0	6.5
Bacterial Spores	1.0	1.0	2.0	6.5
Molds	1.0	1.1	2.2	6.8
LOG OF REDUCTION BASE LINE TOP BRAND 100g washing detergent & 6g PR sanitiser				
Gram positive bacteria	5.3	6.3	7.0	7.0
Gram negative bacteria	5.2	6.3	7.0	7.0
Bacterial Spores	5.0	6.3	7.0	7.0
Molds	5.2	6.2	7.0	7.0

LOG Reduction in Microbiological Analysis

LOG (Logarithmic) Reduction refers to the decrease in the number of microorganisms after a specific treatment, expressed as a factor of 10. It quantifies the efficacy of antimicrobial agents or sterilization processes.

For instance:

- A **1-log reduction** means a 90% reduction (microbial count drops from 10,000 to 1,000).
- A **2-log reduction** corresponds to a 99% reduction (from 10,000 to 100).
- A **3-log reduction** indicates a 99.9% reduction, and so forth.

Higher log reductions signify greater effectiveness in reducing microbial populations.

The presented results clearly show that the addition of just 6 grams of Pikua Rense Sanitizer significantly and statistically improves the efficiency of bleaching, general surfactant washing, and fabric whiteness. It is particularly effective and incomparably more efficient than conventional detergents when it comes to biocidal action against microorganisms present in the laundry or the washing solution.

Improving Dishwashing Machine Performance + IKW Testing

Performance improvement in dishwashing machines involves optimizing cleaning efficiency, energy use, and the care of tableware. The Institute for Detergents, Cleaning, and Washing Agents (*IKW – Industrieverband Körperpflege- und Waschmittel e.V.*) provides standardized methods to assess dishwashing

performance, ensuring consistency and reliability in evaluating detergents and appliances.

Strategies for Improving Dishwashing Machine Performance

1. **Optimizing Detergent Formulation:**
   - **Enzymes:** Enhance the breakdown of proteins, fats, and starches (e.g., protease, lipase, and amylase).
   - **Surfactants:** Improve grease removal and drying performance.
   - **Builders:** Soften water and prevent scaling on dishes and the machine.
2. **Improving Machine Design:**
   - **Water Spray Systems:** Efficiently distribute water and detergent.
   - **Temperature Control:** Allow for optimal cleaning temperatures, typically 50–70°C. o **Energy Efficiency:** Incorporate sensors and energy-saving modes for reduced resource consumption.
3. **Water Softening and Filtration:**
   - Integrated water softeners or external filters prevent mineral buildup and enhance detergent efficacy.
4. **Cycle Customization:**
   - Programs tailored for specific soiling levels and dish types (e.g., delicate glassware, heavily soiled pots).

IKW Dishwashing Test Protocol

The IKW has developed standardized test procedures to evaluate the cleaning performance of dishwashing detergents and appliances. These tests simulate real-world conditions and assess various performance aspects, including stain removal, residue prevention, and overall cleanliness.

Key Elements of the IKW Test:

1. **Standardized Soiled Dishes:**
   Test dishes are prepared with controlled soiling, such as milk, starch, or grease, to mimic household use.
2. **Test Protocol:**
   - Use of a standardized dishwashing machine.
   - Specific water hardness, temperature, and detergent dosages.
   - Defined washing programs to simulate real-life scenarios.
3. **Performance Evaluation:**
   - **Cleaning Efficiency:** Stain removal is visually assessed or measured with instruments (e.g., spectrophotometers).

- **Residue Assessment:** Check for leftover food particles or detergent residues.
- **Glassware Care:** Assess spotting and etching on glass items.

4. **Repeatability and Reproducibility:**
   Results must be consistent across multiple tests and laboratories to ensure reliability.

Applications of IKW Testing

- **Product Development:** Helps manufacturers refine detergent formulations.
- **Quality Assurance:** Ensures compliance with performance standards.
- **Consumer Communication:** Provides data for performance claims, such as
  - "Removes tough grease" or "leaves dishes sparkling clean."
- The Serbian and EU markets feature a top German-made "all-in-one" dishwasher tablet as a leading product.
- Each tablet weighs approximately 20 grams, designed for one washing cycle.
- This tablet was used as a baseline to test the addition of Pikua Rense Sanitizer (PRS).
- Serbian dishwashers typically use a 70°C temperature, while the EU standard is 50°C.
- Tests were conducted using the ECO program at 50°C to reflect EU conditions.
- The goal was to assess PRS's impact on cleaning and sanitation.

Results:

TOP BRAND- 20g tablet on ECO 50C washing program vs. ECO 50C + 5g PRS

	Baseline	Baseline + PRS
OIL/plastic surface	0.15	0.11
Starch/plastic surface	0.15	0.11
OIL/ceramic surface	0.13	0.10
Starch/ceramic surface	0.13	0.10
TEA/ceramic surface	0.12	0.00
Coffie/ceramic surface	0.11	0.00
OIL/steel surface	0.07	0.05
Starch/steel surface	0.07	0.05
Σ/n	**0.12**	**0.07**

BIOCIDAL ACTION

	Base line	Base line+PRS
Gram-positive bacteria	3.6	6.0
Gram negative bacteria	3.5	6.0
Bacterial Spores	1.8	6.0
Molds	1.9	6.0

The presented results clearly show that the addition of just 5 grams of Pikua Rense SANITISER significantly and statistically improves the efficiency of bleaching and general surfactant washing. It is particularly effective and incomparably more efficient than conventional dishwashing detergents when it comes to biocidal action against microorganisms present on the surface of dishes or the washing solution.

Best regards,

Dr A. Radulović, Head of Laboratory

Dr S. Blagojević, General Manager

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Note:

If you have any questions, please do not hesitate to contact us.

We sincerely hope that this information will assist you in your future work.

We would like to take this opportunity to remind you that IOFH (Institute of General and Physical Chemistry – Institut za Opštu i Fizičku Hemiju) has over 63 years of experience in chemical analysis (established on May 6, 1961). The Ministry of Education, Science, and Technological Development of the Republic of Serbia has accredited IOFH as a "Research & Development Institute" in the fields of chemistry and physical chemistry. For more information, please visit www.iofh.bg.ac.rs.

The notification, handling, storage, and measurement of the sample were conducted in accordance with the procedures outlined in the SRPS/ISO 17025:2017 quality system (General requirements for the competence of testing and calibration laboratories).

All equipment used was calibrated in compliance with Serbian regulations, as well as the relevant technical documentation and procedures.

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