The aim of the work was to carry out systematic studies on different “direct” and “indirect” aerobic, as well as anaerobic fermentation, methods for radioactive cotton overalls – radioactive cellulose containing waste (RCCW) reprocessing (bioconversion). Preliminary tests of the cotton degradation with different aerobic fungus species Trichoderma and Aspergillus allowed choosing a strain Trichoderma reesei N/P. Different methods of the strain Trichoderma reesei N/P cultivation and cotton waste pre-treatment were investigated. In having studied the possibility of “indirect” aerobic bioconversion of cotton waste with industrially produced fermentative preparations – cellocom and celloviridine G3x – it was found that to reduce the inhibiting influence of glucose formed as a result of the fermentative hydrolysis of cellulose, yeast inoculation into system (such as the yeast strain Candida tropicalis) was required. In the final part of these studies, experiments on RCCW anaerobic fermentation were carried out. It was established that a semi-continuous process of RCCW anaerobic fermentation with a partial recirculating of non-hydrolysed after-centrifuged solid residue was most effective. The technological flow sheet elaboration and initial data for projecting a pilot plant for the RCCW bioconversion were the main results of the studies conducted.

INTRODUCTION

Increasing non-nuclear radwaste quantities poses a high potential danger for the environment. The ratio of RCCW in the total quantity of such organic radwaste is about 50 – 70 %. The most widely-spread kind of waste is polluted cotton, rags, paper, wood, etc. Nowadays different thermal methods are applied to reduce the volume of RCCW. The thermal treatment of radwaste, in spite of its high efficiency, has considerable disadvantages such as a high process temperature (above 750 °C), high investment, the necessity of off-gas purification from aggressive chemical and radioactive components, and the further conditioning of resultant secondary radioactive waste [1]. Biotechnological methods could be more ecologically clean and less energy expensive for compacting RCCW. At the present time biotechnological methods for the radwaste reprocessing have restricted application. Only biosorption and bioaccumulation of radionuclides from uranium mine waters are currently practiced. Therefore, studies of enzymatic ability of micro-organisms in relation to the organic radwaste is a problem of current interest.

CONCEPT OF RCCW BIOCONVERSION

The concept of radioactive cellulose containing waste biotechnological reprocessing was elaborated on the base of scientific technical and patent references review, as well as the practical experience of MosNPO “Radon” (Moscow Science Industrial Association “Radon”). The concept is represented in Fig.1. The analysis of the given flow sheet (Fig.1) helped reveal the main pa-
rameters of RCCW reprocessing such as: $G_{bg}$ – specific in relation to degradable organic substances gas formation [kg/kg], A – mass share of wet biopulp formed in the separation stage, D – compaction degree of wet biopulp after drying and incineration, $K_d$ – distribution coefficients of radionuclides between a culture liquid and biopulp solids [ml/g]. In regard to the main aim of RCCW reprocessing the following efficiency criteria of studied waste bioconversion methods were developed:

**Waste bioconversion degree** $K_1$ as a relative change of cellulose containing waste mass after reprocessing, %:

$$K_1 = \frac{(m_0 - m_\infty) \cdot 100 \%}{m_0} \rightarrow \max$$  \hspace{1cm} (Eq.1)

**Waste bioconversion rate** $P$ as a relative change of cellulose containing waste mass normalised on the reprocessing duration $\tau$, g/day:

$$P = \frac{m_0 - m_\infty}{\tau} \rightarrow \max$$  \hspace{1cm} (Eq.2)

**Radionuclide concentration in the bioconversion final product** $K_2$ as a relation of radioactivity of the bioconversion final product to the original RCCW:

$$K_2 = \frac{m_\infty C_\infty}{m_0 C_0} \rightarrow \max$$  \hspace{1cm} (Eq.3),

where $C_0$ and $C_\infty$ are specific radioactivity values of the original solid waste coming for reprocessing and the final product of reprocessing, respectively.

**Waste bioconversion intensity** – $T$ as a relative parameter of RCCW bioconversion degree normalized on the reprocessing duration $\tau$, day$^{-1}$:

$$T = \frac{K_1}{\tau \cdot 100 \%} \rightarrow \max$$  \hspace{1cm} (Eq.4).

And finally an integrated **technical and economic criterion** $\Phi$  [2], which allows estimating specific expenses for the RCCW bioconversion, was also derived:

$$\Phi_x = (\Phi_1 + \Phi_2 + \Phi_3 + ... + \Phi_i) \rightarrow \min$$  \hspace{1cm} (Eq.5),

where $\Phi_1$ – expenses for the water and chemicals, $\Phi_2$ – energy expenses (steam, electric power, etc.), $\Phi_3$ – investments, $\Phi_i$ – i-component of other expenses.
EXPERIMENTAL METHODS

The main objects of study used were: cotton (overalls) contaminated with radionuclides $^{60}$Co, $^{90}$Sr, $^{137}$Cs; strains of cellulolytic ferments (enzymes) producers; fungus species *Trichoderma* and *Aspergillus* delivered from the museum of Joint-Stock-Limited Scientific Research Institute «Biotechnology»; fermentative preparations celloviridine G3x (strain producer *Trichoderma viride* 44-11-62/3) and cellocom (strain producer *Aspergillus tericola*) from Privolzhsky biochemical factory; yeast *Candida tropicalis* VSB 928 and *Endomicopsis fitulis* from the collection of SynthezNIIbelok (Moscow); algae biomass (*Elodea canadensis*) from settling ponds of the MosNPO “Radon” site.

Deep aerobic cultivation of the fungi and the yeast in standard nutritious mediums as well as the fermentative hydrolysis of cotton were carried out in a thermostatically controlled shaker under asepsis conditions. Cellulolytic activity $R_1$ of ferments in culture liquids was determined by the Chomodie-Nelson's method. Concentration of the yeast cells was determined in the Goryaev’s chamber. The bioconversion degree of cotton was gravimetrically determined.

Anaerobic fermentation of cotton and algae biomass was conducted in a laboratory plant assembled on the base of fermentation complex «Fermus 3M». During anaerobic fermentation parameters were controlled such as: chemical composition of digested waste, quantity and chemical composition of forming biogas, pH and medium temperature, volatile aliphatic acids (VAA) concentration in the system. The chemical composition of biogas was determined by gas chromatography. The VAA concentration was determined by analysis based on the procedure of VAA distillation with the water steam from the waste samples, followed by titration with an alkali solution in the presence of phenolphthalein.

In bioconversion experiments on simulated radioactive cotton waste (with specific radioac-
tivity of about $10^6$ Bk/kg) distribution coefficients and the degree of removal values for radionuclides $^{60}$Co, $^{90}$Sr, $^{137}$Cs were determined by the radiometric analysis of biomass and culture liquid samples.

**AEROBIC BIOCONVERSION**

In comparative estimation of industrial cellulolitic ferment producers, fungus species *Trichoderma* and *Aspergillus*, a strain of *Trichoderma reesei N/P* having the most degradable activity relative to cotton was chosen. All the rest of the studies on the “direct” aerobic biodestruction of cotton were carried out with the use of the strain *Trichoderma reesei N/P*.

The study of sorption properties of fungi *Trichoderma*, *Aspergillus* and yeast *Candida tropicalis VSB 928, Endomicopsis fitulis* biomass in relation to radionuclides $^{60}$Co, $^{90}$Sr, $^{137}$Cs in static at different pH values and initial specific radioactivity of nutritious medium, different concentrations of microbial suspensions showed that the distribution coefficients ($K_d$) were in the range of $2 - 2000$ ml/g (dried biomass), which was lower than ones for traditionally applied sorbents such as ion-exchange resins, zeolites, synthetic inorganic sorbents, etc.

Increasing the initial specific radioactivity of the nutritious medium or the concentration of microbial suspension caused decreases in $K_d$ which possibly was connected with the surface sorption at three-hours exposure, increasing micro-organism cell coagulation and kinetic limits of radionuclide migration through ion channels of cell membrane into inner cell structures.

Increasing the pH of the medium from 2 up to 10 generally caused an increase in the degree of radionuclide removal from the nutritious medium, which possibly was connected with the increasing radionuclide hydrolysis.

Examination of the kinetics of radionuclides $^{60}$Co, $^{137}$Cs, $^{90}$Sr sorption by the micro-organisms biomass revealed two sorption stages (Fig.2) with different rates, which were possibly caused by the surface sorption on ion-exchange groups of cell membranes (during several hours radionuclides were sorbed up to 40-60% from the equilibrium concentration) and a low rate of radionuclides ionic migration (metabolic inclusion) into inner cell structures.

The experimental results obtained allowed determination of close sorption properties of the tested micro-organisms. In spite of comparatively low values of distribution coefficients the sorption properties of micro-organisms biomass (taking into account its high volume reduction) are enough to remove the radionuclides together with the spent biomass during the RCCW bioconversion. The volume reduction of the examined micro-organisms biomass after drying and subsequent incineration was from 370 up to 1400.
Preliminary tests of fungi *Trichoderma reesei N/P* deep cultivation revealed the possibility of «direct» aerobic bioconversion of cotton in the nutritious medium without any organic additions. However, the efficiency of bioconversion was extremely low. The bioconversion degree in 200 days was about 25%, which was connected with a controlled mechanism of cellulase induction and long term adaptation of the strain to the substratum.

The study of different cultivation methods of fungi *Trichoderma reesei N/P* and substratum pre-treatment to increase the bioconversion efficiency were carried out: the take-off fill up regime of fungi *Trichoderma reesei N/P* static cultivation with returning ½ of non-hydrolysed substratum or culture liquid; thermal or thermochemical pre-treatment of substratum; introduction of the cellulase biosynthesis inductors into the nutritious medium; the use of surface culture fungi *Trichoderma reesei N/P* as an inoculate.

The efficiency assessment of «direct» cotton bioconversion (Table IV) showed that the efficiency considerably increased after the thermal, thermochemical pre-treatment of waste with alkali or acid solutions; the introduction of cellulase biosynthesis inductors into the medium and conducting the take-off fill up bioconversion process. Several static experiments with the substratum thermochemical pre-treated with 3% NaOH aqua solution (0.1 MPa, during 2 hours) and application of surface fungi *Trichoderma reesei N/P* inoculate, in presence of cellulase biosynthesis inductors were carried out. As a result of the experiments 75 % bioconversion degree of cotton was achieved in 15 – 20 days, while the bioconversion rate was up to ~ 0.2 g/day. In the bioconversion of cotton contaminated with radionuclides $^{60}$Co, $^{90}$Sr, $^{137}$Cs the distribution coefficients of radionuclides between the culture liquid and the final product of bioconversion were in the range of 180 - 2150 ml/g (dried residue).

The efficiency of cotton fermentative hydrolysis with the industrial preparations celloviridine G3x and cellocom was greater for celloviridine G3x. Therefore, in further bioconversion experiments only celloviridine G3x was used. The experiments on «indirect» aerobic bioconversion showed that the fermentative activity of celloviridine G3x was inhibited by the hydrolysis product – glucose in concentration 0.06 g/l. On the base of kinetic data of the glucose accumulation in the system kinetic parameters of cotton fermentative hydrolysis $V_m, K_m$ (these are a maximum hydrolysis rate and the Michaelis & Menten’s constant accordingly) and the inhibition constant $K_p=1.72\times 10^{-4}$ M was calculated, which corresponded to reference data.

The data obtained indicated the possibility of increasing the fermentative activity of celloviridine G3x by means of the yeast actively eating the glucose introduced into the system. The experiments (Table I) showed that inoculation with the yeast strain *Candida tropicalis*
considerably decreased the glucose concentration in the system and the fermentative preparation activity increased on the average up to 55%. The use of different compositions of the yeast nutritious medium and ferment concentrations gave the same results. It was experimentally established that the variation of ferment concentration in the system did not change the common shape of the yeast growing curves, the fermentative activity and the glucose accumulation.

**Table I Fermentative activity of celloviridine** (Note: r.u. – relative units).

<table>
<thead>
<tr>
<th>Exposure, day</th>
<th>Celloviridine activity, r.u.</th>
<th>Celloviridine activity in presence of yeast, r.u.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>3</td>
<td>98</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>43</td>
</tr>
</tbody>
</table>

On coming from the obtained data analysis, a laboratory regime of cotton fermentative hydrolysis was elaborated: the concentration of fermentative preparation – 2%, origin substratum – 50 g/l, pH = 5.1 (acetate buffer), t = 40 °C (Table II).

**Table II. Fermentative hydrolysis of cotton by celloviridine G3x.**

<table>
<thead>
<tr>
<th>Exposure, day</th>
<th>Concentration of red sugars, g/l</th>
<th>Concentration of reduction sugars, g/l</th>
<th>Hydrolysis degree, %</th>
<th>Ferment activity, r.u.</th>
<th>Ferment activity, r.u.</th>
<th>LgN</th>
<th>Hydrolysis degree, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.5</td>
<td>8.0</td>
<td>0</td>
<td>140</td>
<td>132</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>12.3</td>
<td>5.7</td>
<td>5</td>
<td>80</td>
<td>74</td>
<td>6.7</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>24.0</td>
<td>3.4</td>
<td>27</td>
<td>48</td>
<td>66</td>
<td>8.6</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>32.0</td>
<td>3.5</td>
<td>37</td>
<td>12</td>
<td>48</td>
<td>9.0</td>
<td>55</td>
</tr>
</tbody>
</table>

The cotton bioconversion rate in the above mentioned conditions of the fermentative hydrolysis without the yeast and with the yeast strain *Candida tropicalis* (∼10⁵ cells/ml) being inoculated was accordingly 0.18 and 0.27 g/day, which was similar to the bioconversion rate achieved in the «direct» aerobic bioconversion.

**ANAEROBIC BIOCONVERSION**

The study of cotton and algae from the settling ponds of MosNPO “Radon” site anaerobic bioconversion efficiency was carried out in the assembled laboratory plant in batch and take-off fill up regimes.

Before loading into the fermenter RCCW was held up under conditions of air oxygen access to activate acidigenes micro-flora until the VAA formed in concentrations of 600 – 1000 mg/l. The conditions of hold up and main anaerobic fermentation of RCCW were similar – pH = 7.0 – 7.4, t = 38 – 42 °C, mixing 50 rpm. It was established that the minimum hold up time for the substrata had to be not less than 6 days. While the RCCW being delayed considerable medium acidulation up to pH=5.7 was observed, which was connected with the VAA concentration increase. The comparative assessment of cotton and algae anaerobic bioconversion in the delay-tank revealed considerable qualitative and quantitative differences. The calculated average values of specific gas formation in the process of organic substances destruction in the delay-tank were
0.00041 ± 0.00004 kg (biogas)/kg (organic substances) per hour and 0.00020 ± 0.00002 kg/kg-h for the algae biomass and cotton respectively, which indicates that the algae biomass was more biologically accessible than the cotton because of the different structural properties and the chemical composition of studied substrata.

After hold up RCCW were loaded into the fermenter. The specific gas formation rate $G_{bg}$ (kg/kg-h) in all the experiments was calculated on the base of biogas chemical composition analysis according to the equation:

$$G_{bg} = \frac{m_{bg} \cdot G_v}{22.4 \cdot m_b \cdot OS}$$

(Eq.6),

where $m_{bg}, m_b$ – average statistic values of the biogas molecular mass [kg/M] and the weight of loaded RCCW [kg]; $OS$ – organic substances contents [% w.], $G_v$ – volume efficiency of gas formation, [l/h].

The chemical composition analysis of biogas in the hold up stage and in the main anaerobic fermentation of cotton and algae biomass indicated approximately similar values (% vol.): in the hold up process CH$_4$ 23.6–33.3%; CO$_2$ 24–27.2%; N$_2$ 26.8–62%; O$_2$ 10–18.9%; NH$_3$, H$_2$S и H$_2$ – less than 1%; in the main process CH$_4$ 40–55%; CO$_2$ 39–46%; N$_2$ 2.8–5.3%; O$_2$ 2.8–6%; NH$_3$, H$_2$S и H$_2$ – less than 1%. Therefore, for calculations the following biogas composition was used (% vol.): for the hold up: CH$_4$ – 33%, CO$_2$ – 27%; for the main process: CH$_4$ – 55%, CO$_2$ – 45%. It was experimentally established that the take-off fill up regime of the substrata reprocessing was characterised with a high biogas release (Table III), but the process productivity (the substratum bioconversion rate) per original waste weight unit was less than in the batch regime, because less organic substances were bioconverted.

The thermochemical pre-treatment of cotton increased its bioconversion rate. Thus, the bio-conversion rate of